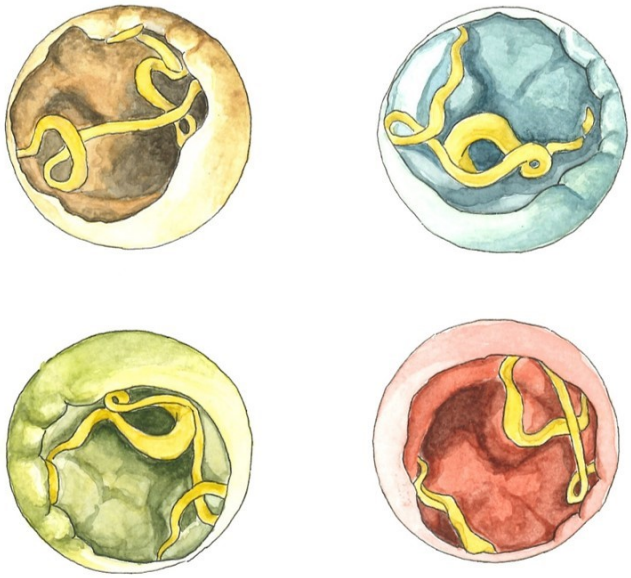
The Costs and Consequences of Parasitism to a Wild Population: An Energetics Approach



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The costs and consequences of parasitism to a wild population: An energetics approach

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Declaration

I hereby declare that this work has been originally produced by myself for this thesis and it has not been submitted for the award of a higher degree to any other institution. Inputs from co-authors are acknowledged throughout.

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Abstract

Parasites are ubiquitous and can have major fitness consequences for their hosts. The effects of most parasites are sub-lethal, with consequences for vital rates such as reproductive output. However, quantifying these costs can be difficult, despite their potential to have large effects on population growth and persistence. Additionally, assessing parasite infections in wild populations is challenging, particularly for endo-parasites where indirect measures can be inaccurate. However, ignoring these effects reduces our understanding of the host's ecology, as we know parasites can impact a large array of fitness-related traits. Here we combine unique methods to directly quantify natural parasite load and an energetics approach, to quantify the costs of parasitism to a wild population of seabirds.

We demonstrate the measurable cost of the intestinal parasites *Contracaecum rudolphii* on reproductive success. The number of fledglings successfully raised by female European shags *Phalacrocorax aristotelis* is negatively related to endo-parasite burden. Furthermore, we demonstrate the mechanistic basis for this finding by developing a calibrated proxy for energy expenditure in this species. We found a positive relationship between a known proxy for resting metabolic rate and parasite burden suggesting maintenance costs increase as a result of parasite infection. There is also a positive relationship between parasite burden and the cost of flight behaviour in females. Individuals with higher flight costs spend a smaller proportion of their time-budget in flight, presumably due to increased flight and maintenance costs associated with higher parasite loads. However, we see no effect of parasitism on daily energy expenditure, suggesting a fixed or optimal energy ceiling and the need to balance increased maintenance costs with reduced activity in this species. Despite previous predictions, these results persist regardless of environmental conditions and mean that we may be greatly underestimating the impact of parasitism on host populations. These costs operate disproportionately on females, possibly due to the greater energetic constraint of reproduction for females than males. This is likely to have important implications for demography and selection.

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Chapter 1

General introduction

Life history trade-offs

The key principle of life-history theory is that organisms are faced with limited resources, such that they need to balance how to allocate their time, energy and nutrients between the many different processes that enhance their fitness (Stearns, 1992; Vedder and Bouwhuis, 2017). Therefore traits or behavioural and physiological adaptions that influence growth, survival and reproductive success may be limited to some degree and traded-off against one another (Agnew et al., 2000; Ricklefs and Wikelski, 2002). As a result, investment in survival or reproduction may vary over time dependant on life-history strategy. An extreme manifestation of this is that , iteroparous species are expected to reduce investment in current reproduction when costs to future reproductive output outweigh the benefits of current reproduction (Cole, 1954; Roff, 2000; Stearns, 1992). Whereas in semalparous organisms where there is only one reproductive event, allocation to reproduction is expected to be fixed at the maximum (Hauteke et al., 2001; Stearns, 1992). Additionally, within iteroparous species trade-offs vary with pace of life (Ricklefs and Wikelski, 2002; Tarka et al., 2018). For instance, long-lived species are expected to invest significant resources in survival and only produce a small number of offspring each year, illustrating trade-offs between maintenance of self and reproduction. For example though short tailed shearwaters (*Puffinus tenuirostris*) have an average breeding life of just under 30 years, they only lay one egg in each annual reproductive event (Moreno, 2003).

Within species or populations, individuals that differ in how they are able to allocate resources to life history traits are likely to have different fitness (Agnew et al., 2000). As a result, in wild populations it is common to find high levels of reproductive skew (Alberts et al., 2003). This describes when a small number of individuals contribute to a large proportion of the population growth and certain individuals are consistently successful whilst others consistently fail. For instance in kittiwakes (*Rissa tridactyla*) only 34% of males that are fledged young go on to breed (Coulson, 1988). In population ecology, we want to understand what drives these individual differences, as it might help us to understand what drives population success or failure (Moreno, 2003). To have

a mechanistic understanding of these drivers however, we need to understand what drives individual successes and failures.

Drivers of individual variation in fitness can be intrinsic (age, quality, sex) or extrinsic (food availability, wind, disease) and can have differential impacts on individuals due to interactions between variables. For example in fulmars (*Fulmarus glacialis*) there is an interaction between past breeding success rate and winter environmental conditions, such that pairs with low past success rate exhibited a sharp decline in breeding success (from 0.4 to 0.2 chicks pair^{-1}) with increasing winter North Atlantic Oscillation (NAO) whereas more successful pairs did not (Lewis et al., 2009). So whilst a population may be exposed to the same driver, individuals may react in different ways. The environment or the ability to gain resources more efficiently can also mediate the role between life-history and fitness, as individuals which encounter more resources in their environment may be able to simultaneously increase their investment in more than one trait, e.g., in barbary macaques (*Macaca sylvanus*) the ability to moderate metabolic needs in relation to environmental and social demands is mediated by nutrition and food availability (Cristóbal-Azkarate et al., 2016). Thus, an individual's environment and its ability to exploit it can have an influential role in the expression of its life-history traits and on its fitness (Agnew et al., 2000).

Sex Differences

A crucial intrinsic driver of variation in individual fitness and behaviour is sex. (Tarka et al., 2018). Natural and sexual selection are expected to act differently on males and females often leading to sexual dimorphism in life-history traits (Hämäläinen et al., 2018; Parker, 1979). Indeed, life-history traits, commonly differ between the sexes across the animal kingdom (Clutton-Brock, 1991; Tarka et al., 2018; Trivers, 1972). These range from differences in lifespan, rate of growth, reproduction, and aging (Bonduriansky et al., 2008; Murgatroyd et al., 2018a) and physiological traits (Bize et al., 2005; Poulin, 1996; Roved et al., 2017), as well as differences in behaviour (Patrick and Weimerskirch, 2014; Schuett et al., 2010).

Typically, these differences are more apparent or measureable during the reproductive period. For instance in sexually reproducing species there are significantly different energetic costs of gamete production in males and females (Hayward and Gillooly, 2011). Estimates of the cost of egg production in birds ranges from 20% to 200% of Basal Metabolic Rate (BMR) compared to an estimated 0.4% BMR for the cost of sperm production (Hayward and Gillooly, 2011). These maintenance costs and metabolic rates vary between sexes across a large number of taxa (Burton et al., 2011) and can result in different energetic demands to the sexes (Drent and Daan, 1980; Hicks et al., 2018; Lewis et al., 2015). These sex specific differences in energy use and allocation to reproduction (Clutton-Brock, 1991; Kokko and Jennions, 2003; Trivers, 1972) can result in different energetic trade-offs (Hicks et al., 2018; Korpimaki et al., 1993; Reed et al., 2008b) and lead to, or be a consequence of, sex specific foraging behaviours. Sex differences in foraging behaviours as well as interactions between fitness related foraging strategy, personality and sex have been found in a broad range of sexually dimorphic as well as monomorphic species (Patrick and Weimerskirch, 2014) for a review in seabird species see (Lewis et al., 2002).

Different trade-offs and life-history strategies can lead to differential investment in reproduction and immune function which can have consequences for parasitism defence (Saino et al., 2003). Indeed, in a large number of species, a major explanation for sex-specific mortality is sex differences in immune defence, with males possessing smaller immune defence organs and mounting weaker humoral and cellular immune responses in comparison with females (Bize et al., 2005; Grossman, 1985; Klein, 2004; Moller and Saino, 2004). Across many taxa males are also more susceptible to parasites due to immunological differences; as well as hormonal differences, whereby androgens can mediate immune responses (Grossman, 1985; Klein, 2004; Poulin, 1996).

Parasitism as an ecological and evolutionary driver

It is well known that parasites can have major detrimental fitness consequences on their hosts, e.g. through reduction of host condition, reduction in reproductive success and increase in infant winter mortality (Arnold and Anja,

1993; Booth et al., 1993; Gooderham and Schulte-Hostedde, 2011; Reed et al., 2008b). Such consequences may arise due to multiple costs imposed by parasites, such as immune or stress responses, the direct extraction of resources from their hosts, and tissue damage caused by the parasite (Martin et al., 2003; Sheldon and Verhulst, 1996; Smyth and Drea, 2016). These costs can result in changes in behaviour, condition, fecundity, survival and future reproduction in many species including fish, ungulates, passerine birds and seabirds (Binning et al., 2012; Bize et al., 2004; Reed et al., 2008b; Sheldon and Verhulst, 1996; Stien et al., 2002). Due to the numerous costs of parasitism, in long-lived iteroparous species, where survival rate contributes more to fitness than in short-lived species, individuals should be selected to allocate a strong immune response to combat damage from parasites in order to protect future reproductive success (Lochmiller and Deerenberg, 2000). However, as activating an immune response is energetically costly (Ilmonen et al., 2000; Råberg et al., 2000), this may cause trade-offs between immune response and other fitness related activities associated with immediate reproduction. By altering demographic traits, parasitism can therefore impact on population-level processes. This has been demonstrated by parasite removal experiments which disrupt population cycling in red grouse (*Lagopus lagopus scoticus*) and increase the probability of calf production in reindeer (*Rangifer tarandus*) by up to 0.13 and increase fecundity in mountain hares (*Lepus timidus*) (Albon et al., 2002; Hudson, 1998; Newey and Thirgood, 2004).

Parasitic costs can lead to lethal outcomes and influence demography through survival (Lehmann, 1993). However most parasites will inflict sub-lethal effects which have the potential to hugely alter individual success. Yet it is hard to quantify sub-lethal costs to individuals, especially in wild populations (Watson, 2013). Furthermore the cost of natural parasitism to individual fitness has yet to be established or quantified in wild populations. This is mostly due to the difficulty of measuring sub-lethal fitness costs and accurately measuring parasite loads in wild populations. Endo-parasites in particular can be hard to quantify accurately, as traditional methods such as faecal egg count (FEC) have been shown to significantly underestimate prevalence, by up to 60% compared

with the endoscopy method (Granroth-Wilding et al., 2016). Yet such macro-parasites are thought to be particularly costly to the host (Albon et al., 2002). As such, the effects of parasites are rarely incorporated into demographic models, leading to potentially erroneous conclusions about factors affecting reproduction (Binning et al., 2017).

It is thought that population regulation by parasitism under natural conditions may only occur in extreme scenarios and thus fitness effects of parasites may only occur in the wild when hosts are perturbed by other factors (Tompkins et al., 2011). The effects of parasitism are also known to be condition dependent (Bize et al., 2010; Reed et al., 2008b), thus may have differential individual impacts due to interactions between extrinsic and intrinsic variables (Daunt et al., 2014; Lewis et al., 2009). This can be a sign of quality or different investment priorities, whereby individuals have different tolerances to parasitism or invest varying amounts of resources to an immune response. This is speculative however and very little is known about individual host responses to parasitism (Granroth-Wilding et al., 2016). It is therefore important to quantify the cost of parasitism alongside other drivers of fitness to investigate how intrinsic and extrinsic factors may interact to drive the success of populations. One way of doing this is to use a common currency in which fitness costs from multiple drivers can be disentangled, quantified and compared between individuals. One such common currency is energy.

Energy expenditure and fitness

Energy is a fundamental resource needed for survival and reproduction and is a central currency in the behaviour and physiology of animals (Butler et al., 2004; Welcker et al., 2010). Energy is required for the physiological processes that generate behaviour, build new biomass (e.g. growth and repair, offspring production), and for the many other physiological systems that support life (e.g. digestion, mounting an immune defence) (Biro and Stamps, 2010). Individuals have finite energy to allocate to fitness (Drent and Daan, 1980; Elliott et al., 2014b) and hence life history is constrained by energetics (Brown et al., 2004).

Energy expenditure can be limited intrinsically, by physiological properties of the animal constraining its capacity to process energy (metabolic,

thermoregulatory or digestive limits) (Krol et al., 2007; Welcker et al., 2010) or extrinsically by the amount of energy available in the environment or by the ability of animals to extract energy from the environment (Speakman et al., 2003; Tinbergen and Verhulst, 2000; Welcker et al., 2010). Additionally energy can be limited strategically based on trade-offs between elevated metabolism and survival (Elliott et al., 2014b). As energy is so crucial to all physiological processes it is helpful as a currency to understand the trade-offs associated with life-history strategies and individual differences in fitness. By quantifying traits in a common currency, we can gain more insight into how energy is allocated and partitioned to different behaviours and processes to understand how life-history decisions are made (Green et al., 2009; Tomlinson et al., 2014). Therefore a mechanistic understanding of an organism's energy requirements allows us to understand the interaction between physiological constraints and environmental limitations (Tomlinson et al., 2014).

In free-ranging animals, it is hard to accurately measure energy expenditure in natural settings, often described as measurement of 'field metabolic rate' (FMR) or 'daily energy expenditure' (DEE). DEE can be defined as the cost of existence for an individual in its natural environment (Tomlinson et al., 2014) and is made up of metabolic scope (the energy available to allocate to any fitness related activities) and basal or resting metabolic rate (RMR) (Mathot and Dingemanse, 2015). For endotherms, RMR represents the minimum energetic cost of living during thermo-neutral rest (Mathot and Dingemanse, 2015; Welcker et al., 2015) and therefore largely represents the cost of self-maintenance, including immune activity (Burton et al., 2011).

The two main techniques for measuring DEE in the field are the doubly labelled water method and heart-rate method (Butler et al., 2004; Green, 2011; Speakman, 1997). The doubly labelled water (DLW) method provides a single estimate of the rate of oxygen consumption ($\dot{V}o_2$) over an integrated time period (Butler et al., 2004; Halsey et al., 2008). The heart rate method (f_H) provides high-resolution estimates of energy expenditure in free living animals based on the physiological relationship between f_H and $\dot{V}o_2$. However, f_H must be calibrated

and it often involves implantation of loggers (Butler et al., 2004; Green, 2011; Green et al., 2009).

More recently, a third technique has been developed using accelerometers to measure the Overall Dynamic Body Acceleration (ODBA) of an organism (Halsey et al., 2011a; Wilson et al., 2006a). This assumes that the movement costs of an animal constitute the majority of energy expended (Karasov, 1992); and therefore, body acceleration should correlate with energy expenditure and provide an index of $\dot{V}o_2$ (Elliott et al., 2013a; Gleiss et al., 2011; Halsey et al., 2011a; Wilson et al., 2006a). This is evidenced by significant calibration relationships between $\dot{V}o_2$ and ODBA across a number of taxa in controlled conditions (Halsey et al., 2008; Halsey et al., 2009). Additionally, accelerometer data can provide behavioural information (Yoda et al., 2001), presenting an opportunity to estimate the energetic cost of different behaviours in free-living individuals as well as providing behavioural mechanisms by which animals alter energy expenditure (Halsey et al., 2011a; Wilson et al., 2006a).

RMR is usually measured under controlled conditions (Mathot and Dingemanse, 2015) and as a result is difficult to measure in the field, and so thyroid hormone concentrations are increasingly being used as a proxy (Chastel et al., 2003a; Elliott et al., 2013b; Welcker et al., 2013). Triiodothyronine hormone (T3) especially is considered one of the major controllers for the regulation of tissue oxygen consumption and metabolic activity in endotherms (Blévin et al., 2017). Studies carried out both in the laboratory and the field show close relationships for a range of species and life-history strategies, with coefficients of determination ranging from 0.36 - 0.60 (eg Chastel et al., 2003a; Elliott et al., 2013b; Vézina et al., 2009; Welcker et al., 2013; Zheng et al., 2014). Using T3 hormone as a proxy for RMR allows for estimation of individual measurements without the confounding effect of stress of capture associated with respirometry (Welcker et al., 2015).

Study species and system

Shags and cormorants (family *Phalacrocoracidae*) are piscivorous foot propelled diving seabirds with a broad global distribution (Enstipp et al., 2007; Wanless and Harris, 1997; White et al., 2011). They carry only small fat reserves

and their flight costs are high, meaning they are energetically constrained and need to balance energy requirements on a daily basis (Grémillet et al., 2003). This makes them very sensitive to any factors affecting the cost of their foraging or ability to assimilate energy and as such they are susceptible to population crashes in winter when foraging costs increase due to wind and extreme weather events (Daunt et al., 2006; Frederiksen et al., 2008; Grémillet et al., 2003; Lewis et al., 2015). As such Shags display large variation between years in survival, which is often referred to as "boom–bust dynamics" (Frederiksen et al., 2008). Variation in environmental factors such as food availability and sea temperature can also drive reproductive success throughout their lifetime (Burthe et al., 2012). Thus factors affecting individual success have the potential to have large population consequences, and so parasitism effects could have the potential to rapidly be reflected at the population level.

Populations of European shags (*Phalacrocorax aristotelis*) are susceptible to nematode gastro intestinal parasites, in particular anisakid nematodes *Contracaecum rudolphi*. Shags become infected with third stage larvae via their fish diet. Larval worms moult to become sexually mature adults which attach to the lining of the proventriculus and lower oesophagus in the final seabird host (Abollo et al., 2001; Burthe et al., 2013). Effects are usually sub-lethal but they compete with the host for nutrients and can initiate costly immune responses (Abollo et al., 2001). Almost all adults host worms, but there is large variation in burdens between individuals (Abollo et al., 2001; Burthe et al., 2013). In an experiment using parasite-treated and control adult shags, intestinal parasites were found to constrain the ability of mothers, in particular, to rear more expensive male offspring (Reed et al., 2008b). However, we do not yet understand the mechanism by which this operates.

European Shags are an excellent model system since they are large enough to carry biologging devices (e.g. accelerometers) and their parasite burdens can be assessed using the latest endoscope methodologies (Burthe et al., 2013). Shags are also long-lived with long breeding lifespans, meaning they may be more likely to alter their reproductive investment to parasitism than short lived

species (Lochmiller and Deerenberg, 2000). Breeding adult shags are central place foragers commuting between the nest site and foraging grounds, experiencing high energetic costs of foraging activities (Enstipp et al., 2005). As such, they are a unique study species to test the effects of parasitism on the cost and allocation to key foraging activities. Furthermore, the Shag population on the Isle of May have been intensively studied since the 1980s, providing an extensive long-term dataset on demography, parasitism and behaviour at the individual level (Daunt et al., 2014). Having detailed information on individuals within the population is really powerful, as it allows intrinsic factors such as age and sex, which we know to affect parasite load and life-history decisions, to be taken into account.

Thesis outline

In this thesis I investigate the costs and consequences of natural endo-parasite burdens to European shags. I use an energetic framework to enable the quantification of the costs of parasitism to behaviour-specific components of individual energy budgets as well as the maintenance costs of an immune response. To understand the consequences of these costs I use long-term life-history data to link parasite load to individual breeding success.

Chapter 2 sets out to establish a behaviour-specific calibration for energy expenditure using Overall Dynamic Body Acceleration (ODBA) in free living shags. We simultaneously deployed accelerometers and heart rate loggers in adult shags to compare the accelerometry method against the more established Heart rate method for estimating energy expenditure. We develop behaviour-specific calibrations for estimating energy expenditure from ODBA to use in the other chapters in this thesis. This chapter has been published in the Journal of Experimental Biology

Chapter 3 uses the calibrations established in Chapter 2 to quantify the impact of parasitism on energetic and behavioural costs in shags. We use accelerometry to estimate energy expenditure and time budgets for flying, diving and resting behaviours in adults with varying parasite loads across multiple breeding seasons. This aims to attempt to understand the energetic and behavioural mechanisms by which parasitism may alter individual fitness

in wild populations. This chapter has been published in Proceedings of the Royal Society B, Biological Sciences.

Chapter 4 uses a hormonal proxy (Thyroid hormone) for Resting Metabolic Rate (RMR) to understand how maintenance costs may vary under varying parasite loads assuming that parasitism incurs immune costs to the host which are reflected in RMR. We also use accelerometry derived estimates of Daily Energy Expenditure (DEE) in conjunction with our RMR proxy to understand the energy management strategy of shags and the role of parasitism in mediating the trade-off between allocation of energy to self-maintenance and activity.

Chapter 5 attempts to understand and quantify how natural parasite burdens may impact upon the breeding success of individuals via sub-lethal reduction in fitness. We incorporate individual and environmental variability by using seven years of individual reproductive success and parasite load data to quantify the cost of parasitism to the fledgling success of males and females.

Chapter 6 aims to summarise the main findings and key approaches used and put them into context of life-history and the ecological physiology literature as well as looking forward to new emerging questions from this work.

Statistical approach

I take two statistical approaches within this thesis; in chapters 2, 3 and 4 I take an information criterion approach as the questions in these chapters involve the prediction of estimates from the consequent models. In Chapter 5 however I take a frequentist approach due to the specific hypotheses being tested in this chapter as well as the more easily interpretable results of this approach.

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Chapter 2

Validating accelerometry estimates of energy expenditure across behaviours using heart rate data in a free-living seabird

Validating accelerometry estimates of energy expenditure across behaviours using heart rate data in a free-living seabird

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J.G, S.B and F.D collected the data. C.B aided in preliminary data processing of the heart rate data for this study. O.H processed the accelerometry data, conducted the statistical analyses and wrote the manuscript. A.B provided statistical advice. All authors (O.H, S.B, F.D, A.B, C.B, and J.G) contributed to interpreting results and improvement of this paper.

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Abstract

Two main techniques have dominated the field of ecological energetics, the heart-rate and doubly labelled water methods. Although well established, they are not without their weaknesses, namely expense, intrusiveness and lack of temporal resolution. A new technique has been developed using accelerometers; it uses the Overall Dynamic Body Acceleration (ODBA) of an animal as a calibrated proxy for energy expenditure. This method provides high resolution data without the need for surgery. Significant relationships exist between rate of oxygen consumption ($\dot{V}o_2$) and ODBA in controlled conditions across a number of taxa; however, it is not known whether ODBA represents a robust proxy for energy expenditure consistently in all natural behaviours and there have been specific questions over its validity during diving, in diving endotherms. Here we simultaneously deployed accelerometers and heart rate loggers in a wild population of European shags (Phalacrocorax aristotelis). Existing calibration relationships were then used to make behaviour-specific estimates of energy expenditure for each of these two techniques. Compared against heart rate derived estimates the ODBA method predicts energy expenditure well during flight and diving behaviour, but overestimates the cost of resting behaviour. We then combine these two datasets to generate a new calibration relationship between ODBA and $\dot{V}o_2$ that accounts for this by being informed by heart rate derived estimates. Across behaviours we find a good relationship between ODBA and $\dot{V}o_2$. Within individual behaviours we find useable relationships between ODBA and $\dot{V}o_2$ for flight and resting, and a poor relationship during diving. The error associated with these new calibration relationships mostly originates from the previous heart rate calibration rather than the error associated with the ODBA method. The equations provide tools for understanding how energy constrains ecology across the complex behaviour of free-living diving birds.

Introduction

Energy is a central currency in the behaviour and physiology of animals (Butler et al., 2004). Individuals have a finite amount of energy to allocate to maximising fitness and hence life history is constrained by energetics (Brown et al., 2004). Such constraints can result in trade-offs between survival and reproduction (Brown et al., 2004; Halsey et al., 2009). By understanding energetics, we are able to gain a more mechanistic understanding of these trade-offs. To achieve this, we need to quantify how energy is allocated and partitioned to different behaviours and processes to understand how life-history decisions are made (Green et al., 2009; Tomlinson et al., 2014), and improve the predictive power of species distribution or population dynamic models (Buckley et al., 2010).

The two main techniques for measuring energy expenditure in the wild are the doubly labelled water method and heart-rate method (Butler et al., 2004; Green, 2011). The doubly labelled water method provides a single estimate of the rate of oxygen consumption ($\dot{V}o_2$) over the course of the experiment with no frequency or intensity information (Butler et al., 2004; Halsey et al., 2008). The doubly labelled water technique is a widely accepted method due to extensive validations and widely used due to the relative ease of implementation (Butler et al., 2004; Halsey et al., 2008). The heart rate method relies on the physiological relationship between heart rate (f_H) and $\dot{V}o_2$, and can provide high-resolution estimates of energy expenditure in free living animals. However, the f_H method must be calibrated in controlled conditions and it often involves invasive surgery, particularly for aquatic animals, which can be costly to the animal (Butler et al., 2004; Green, 2011; Green et al., 2009). Information on the behavioural mode of the individual is not inherent or easily estimated in either the doubly labelled water or heart rate methods. Therefore, without extra assumptions (e.g. Portugal et al. 2012; Green et al. 2009) or secondary loggers they have limited capacity to estimate behaviour specific energy expenditure.

Recently, a new technique has been developed using accelerometers to measure the Overall Dynamic Body Acceleration (ODBA) of an animal as a proxy for energy expenditure (Halsey et al., 2011a; Wilson et al., 2006a). Energy costs of animal movement often constitute the majority of energy expended (Karasov,

1992); therefore, body acceleration should correlate with energy expenditure and provide an index of $\dot{V}o_2$ (Elliott et al., 2013a; Gleiss et al., 2011; Halsey et al., 2011a; Wilson et al., 2006a). Significant calibration relationships exist between $\dot{V}o_2$ and ODBA across a number of taxa in controlled conditions (Halsey et al., 2008; Halsey et al., 2009). Additionally, accelerometer data can provide high resolution behavioural information (Yoda et al., 2001), presenting an opportunity to estimate the energetic cost of different behaviours in free-living individuals (Halsey et al., 2011a; Wilson et al., 2006a). Due to the miniaturisation of accelerometer loggers and their ability to collect high-resolution data without surgery, the use of this technique in the field of ecological energetics has grown substantially in recent years, with research focussing particularly on marine vertebrates (Halsey et al., 2009; Tomlinson et al., 2014; Wilson et al., 2006a). However, muscle efficiency may vary across locomotory modes, meaning the relationship between oxygen consumption and accelerometry may also differ among modes (Gómez-Laich et al., 2011). In particular, there have been concerns over the use of ODBA as a proxy for energy expenditure during diving, given equivocal results across several air breathing species in captive and semi-captive conditions (Fahlman et al., 2008a; Fahlman et al., 2008b; Halsey et al., 2011b). This may be particularly problematic in volant birds since they operate in both air and water, and, the higher density and hence resistance of water compared to air can dampen movements at the same level of power output (Gleiss et al., 2011; Halsey et al., 2011b). The indirect metabolic costs of hypothermia may also complicate the relationship (Enstipp et al., 2006). These findings contrast with studies which have established the effectiveness of heart rate as a proxy for energy expenditure under similar conditions (Green et al., 2005; White et al., 2011).

As with the heart rate method, calibrations of ODBA are required before it can be used to estimate energy expenditure. However, calibrations performed in controlled environments such as treadmills or dive tanks, may cause problems for extrapolation to free-living animals, as they do not fully cover the scope of complex natural behaviours (Elliott et al., 2013a; Gómez-Laich et al., 2011; Green et al., 2009). Given the importance of quantifying energetic cost of behaviours

to understand the fitness consequences in wild populations, it is crucial to validate the accelerometry technique across the natural range of locomotory modes in free-living animals. Validations exist using the doubly labelled water method which shows that ODBA predicts daily averages of energy expenditure (Elliott et al., 2013a; Jeanniard-du-Dot et al., 2016; Stothart et al., 2016). However, as the accelerometry technique develops and is now able to discern and estimate energy expenditure across fine scale behaviours, it is timely to validate these measurements with a technique with equally high resolution (Green et al. 2009).

In this study, we aimed to validate the accelerometry technique against the more established heart rate method in wild free-living European shags *Phalacrocorax aristotelis*, a diving seabird species. Since calibration relationships exist between $\dot{V}o_2$ and ODBA and f_H for this genus (White et al., 2011; Wilson et al., 2006a), we are able to directly compare these estimates in a free-ranging bird for the first time (Weimerskirch et al., 2016). We simultaneously measured heart rate and acceleration across known behavioural states, including resting, flight and diving, at high temporal resolution, across the natural behavioural range of this diving bird. This allowed us to address the following questions: 1. When using calibration relationships developed in the laboratory, how do estimates of $\dot{V}o_2$ derived from ODBA compare with those derived from f_H at fine temporal scales across behaviours? 2. Is there value in combining what we know from f_H-derived estimates of $\dot{V}o_2$ to generate calibration relationships to predict behaviour specific estimates of ODBA-derived $\dot{V}o_2$?

Materials and methods

The study was carried out on the Isle of May National Nature Reserve, south-east Scotland (56°11'N, 2°33'W) during the breeding season of 2011. European shags are medium sized foot propelled diving seabirds that feed benthically on small fish such as sandeel (*Ammodytes marinus*) and butterfish (*Pholis gunnellus*)(Watanuki et al., 2005; Watanuki et al., 2008). During chick rearing they typically make 1-4 foraging trips a day (Sato et al., 2008; Wanless et al.,

1998). Twelve adult female European shags were captured on the nest during incubation using a crook on the end of a long pole. Females were used to reduce inter-individual variation in $\dot{V}O_2$ estimates. Birds were anesthetised by a trained veterinary anaesthetist (using isoflurane inhaled anaesthesia) to allow for the implantation of combined acceleration and heart-rate logger devices. This procedure took approximately 60 minutes and once recovered, birds were kept for approximately 40 minutes before being released. Continuous observation of four birds in the field suggested birds resumed normal behaviour in 24 hours. Eleven of the 12 instrumented birds were recaptured in the same manner, approximately 35 days later, and anesthetised to remove the logger. The 12th individual evaded capture due to a failed breeding attempt and was recaptured and its logger removed in the 2012 breeding season. Ten birds fledged at least 1 chick (one brood failed in a storm) in 2011 and the 12th bird successfully bred in 2012. A binomial GLM was conducted to compare the breeding success of instrumented birds (n=12) with uninstrumented birds (n=195). Instruments had no significant effect on breeding success ($Z=0.77$, $p = 0.44$, $df = 205$). Eight of the twelve loggers were fully functional and recorded from 4 to 33 days of data, totalling 162 days of activity during the breeding season. All studies were carried out with permission of Scottish National Heritage and under home office licence regulation.

Instruments

Loggers were custom-built and measured heart rate (f_H), tri-axial acceleration, depth and temperature. The data loggers (50 mm with a diameter of 13 mm, 25g; 1.6% of the body mass of the sampled individuals, mean (± SD) mass = 1561±38) and were programmed to store acceleration at 50 Hz, and depth and temperature with a resolution of 0.02 m and f_H every second. Devices were sterilised by immersion in Chlorhexidine gluconate in alcohol and rinsed in saline.

Data preparation

Coarse scale behaviours were categorised from accelerometer data to differentiate between diving, flying and resting (the three main activities of shags) in two steps. First, ethographer software package (Sakamoto et al., 2009) from IGOR Pro (Wavemetrics Inc., Portland, OR, USA, 2000, version 6.3.5) was used to assign data as diving or non-diving behaviour through supervised cluster analysis using k means methods on the depth trace (Sakamoto et al., 2009). Second, the remaining accelerometer data was assigned as either flight or resting behaviour (either at sea or on land) using frequency histograms of accelerometer metrics to discriminate between these two coarse scale behavioural states (Collins et al., 2015a). Histograms of standard deviation of the heave axis and pitch (the angle of the device and therefore also of the bird in the surge axis) calculated over 60 seconds were used to discriminate between flight and rest behaviour:

$$\text{Pitch} = \text{Arctan}\left(\frac{x}{(Y^2+Z^2)^{\frac{1}{2}}}\right) * \left(\frac{180}{\pi}\right) \quad (1.)$$

Where X is acceleration (g) in the surge axis, Y is acceleration (g) in the sway axis, and Z is acceleration (g) in the heave axis.

Overall dynamic body acceleration (ODBA) was calculated by first smoothing each of the three acceleration channels with a running mean to represent acceleration primarily due to gravity. In our study, the running mean was 1s (i.e. 50 data points) as in Collins et al., 2015. The smoothed value was then subtracted from the corresponding unsmoothed data for that time interval to produce a value for g resulting primarily from dynamic acceleration (Wilson et al., 2006a). Derived values were then converted into absolute positive units, and the values from all three axes were summed to give an overall value for dynamic acceleration experienced. Estimates of the rate of oxygen consumption (ml min^{-1}), were derived from values of both heart rate and ODBA using calibrations conducted in the laboratory on a congeneric species of seabird, the great cormorant *Phalacrocorax carbo* see appendix 1 for calibration equations (White et al., 2011; Wilson et al., 2006a). Great cormorants and European shags are very similar in their geographical ranges, behaviour and physiology thus we feel

confident that the original calibrations can be used for the European shag. All estimates were 'whole animal 'since both calibration procedures took intra-individual variation in body mass into account. Locomotory modes included resting, walking and diving during heart rate calibrations and walking and resting during the ODBA calibration. There are no empirical measurements of $\dot{V}O_2$ for flight in great cormorants. However, previous estimates of $\dot{V}O_2$ during flight from heart rate are comparable to modelled estimates, suggesting that this f_H- $\dot{V}O_2$ relationship is robust for flight.

Finally, a dataset was created containing values of ODBA, f_H and both estimates of $\dot{V}O_2$ averaged across each behavioural period per individual, defined as a period of any length of one of the three behavioural states before the next behavioural states begins. We did not constrain the duration of behavioural periods, but took the duration of each period into account during analyses. This dataset was cropped to three full 24 hour days during incubation for each individual to keep the duration of data consistent across individuals.

Data analysis

There were two objectives in the analysis, firstly to compare ODBA derived estimates of $\dot{V}O_2$ with f_H derived estimates of $\dot{V}O_2$ to investigate if a one-to-one relationship exists between these two methods (question 1) and secondly to establish whether a relationship between ODBA and ƒH derived $\dot{V}O_2$ would allow improved prediction of behaviour specific estimates of $\dot{V}O_2$ from accelerometry at a fine temporal resolution (question 2).

To address question 1 (How do ODBA and f_H derived estimates of $\dot{V}O_2$ compare), we modelled f_H derived $\dot{V}O_2$ using linear mixed effects models (LMMs) using the lme4 package in R (Bates et al., 2014; R Core Team, 2015). ODBA derived $\dot{V}O_2$ and behavioural state were explanatory variables and we controlled for variation between birds by including individual as a random factor. We fitted models containing all possible combinations of the fixed effects, including models with and without interaction terms (see table 2.1). Within each model observations were weighted by the duration of each behavioural bout divided by the sum of the duration of behavioural bouts for each individual for that behaviour to

provide higher weighting to behavioural bouts that are carried out for a longer duration which represent more generalised behaviours. This ensured that short-lived and/or infrequently expressed behaviours were not over represented.

To address question 2 (generating calibration relationships between ODBA and f_H derived $\dot{V}o_2$) we created a second set of LMMs. The model structure was the same as before, except that, in the fixed effects part of the model, ODBA derived $\dot{V}o_2$ was now replaced with ODBA itself.

Table 2.1. Model terms and the corresponding AIC values for GLMMs comparing $\dot{V}o_2$ derived from the heart rate and accelerometry techniques.

Model	Δ AIC	k
Behaviour * $\dot{V}O_2$ (ODBA)	0	8
Behaviour + $\dot{V}O_2$ (ODBA)	63.18	6
$\dot{V}O_2$ (ODBA)	197.76	4
Behaviour	433.01	5

To address question 2 (generating calibration relationships between ODBA and f_H derived $\dot{V}o_2$) we created a second set of LMMs. The model structure was the same as before, except that, in the fixed effects part of the model, ODBA derived $\dot{V}o_2$ was now replaced with ODBA itself.

In both model sets, model selection was based on Akaike's information criterion (AIC), which penalises the inclusion of unnecessary parameters in models (Burnham and Anderson, 2001). The model with the lowest AIC is usually chosen to be the 'best' model, but models within two Δ AIC of the lowest value are generally considered to have similar empirical support to that of the best model. R squared values were calculated using the MuMIn package in R.

Both ODBA and f_H are often used to make qualitative comparisons of energy expenditure between e.g. behavioural states or individuals (e.g. Angel et al. 2015; Green et al. 2009). As we aimed to be able to make quantitative estimates and comparisons of $\dot{V}o_2$ using ODBA (question 2) we needed to incorporate the error associated with the conversion from f_H to $\dot{V}o_2$ into our predictions. To quantify this we developed a bootstrapping approach, which we implemented separately for each behavioural state. For each state we used a fitted model of f_H as a function of ODBA to simulate 100 possible f_H values for given values of

OBDA: these f_H values were drawn from a normal distribution with mean equal to the estimated value of f_H (based on the fitted model) and standard deviation equal to the standard error of the estimate (SEE) that was produced by the fitted model. For each of these f_H values we then simulated 100 values of VO_2 using the fitted equation, and associated SEE, from White et al. (2011). This gives a total of 10000 simulated values of VO_2 for each value of ODBA. We took the mean of these values to be our estimate for the value of VO_2, for each value of OBDA, and used the 2.5% and 97.5% quantiles to give us the associated 95% confidence limits. Both sets of SEE calculations assumed 100 measurements of ODBA from each of 10 individuals; these were assumed to be a typical sample size of individuals and average number of ODBA measurements per individual. These error distributions are calculated to enable the calibrations to be used with quantifiable error associated with the predictions. See Green et al., (2001) for a full description of how SEE calculations are made.

Results

Comparison of oxygen consumption estimates

There was a positive relationship between f_H-derived $\dot{V}o_2$ and ODBA derived $\dot{V}o_2$ (Figure 2.1.). The best model included an interaction between ODBA derived VO_2 and behaviour (Table 2.1) suggesting a difference among behaviours in the relationship between oxygen consumption estimates. Pairwise comparisons revealed differences among all three behaviours in the relationships between the estimates of $\dot{V}o_2$ made using the two techniques. The best overall model was a good fit (marginal R2 = 0.70); however R2 for behaviour specific relationships were much lower (Table 2.2). When the behaviours were considered individually, there was a positive relationship for flying and resting and but no relationship for diving (Table 2.2). Estimates of $\dot{V}o_2$ from both f_H and ODBA showed considerable variability but sat close to the line of equality for flight and diving behaviour. However, ODBA based estimates of $\dot{V}o_2$ were consistently greater than those estimated by f_H (Figure 2.1.). There was relatively little variability in ODBA derived $\dot{V}o_2$ during resting behaviour, this can

be attributed to similarly little variability in raw ODBA values (Supplementary materials Figure S2.1.).

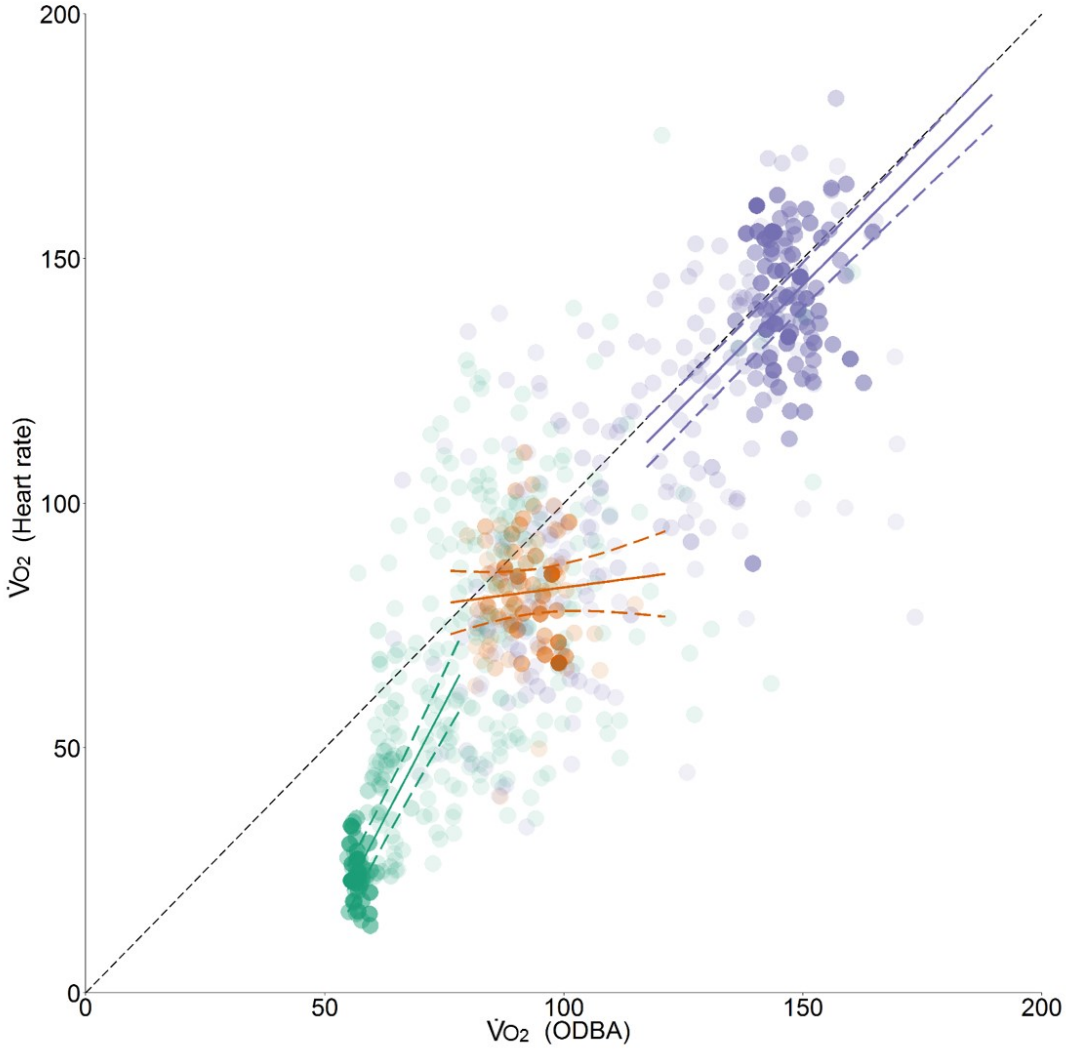

Figure 2.1. The relationship between the two methods for predicting $\dot{V}O_2$ (ml min^{-1}) across different behavioural states. The dotted line represents equality between the two methods. Behaviour specific regression relationships (solid line) and 95% confidence intervals (dashed lines) for each behaviour (resting in green, diving in orange and flying in purple) are shown. Points vary in transparency according to the duration of time represented by each behavioural bout. The horizontal and vertical range of the regression lines indicates data points encompassing 99% of the entire duration of time spent in each behaviour.

ODBA as a predictor of VO2

When using ODBA as a predictive tool for estimating energy expenditure there was a positive relationship between ODBA and f_H derived $\dot{V}O_2$. The best model fitted an interaction between ODBA and behaviour (Table 2.3). Examination of behaviour specific relationships (Figure 2.2) suggest that ODBA is a useable proxy of $\dot{V}O_2$ during flying and resting, but a poor proxy for diving (see Table 2.4

for behaviour specific predictive equations). When accounting for the residual error associated with the f_H $\dot{V}o_2$ calibration, it is evident that a large amount of error is associated with the laboratory calibration between f_H and $\dot{V}o_2$. Indeed, most of the uncertainty in predicting heart rate derived $\dot{V}o_2$ from ODBA arises from the uncertainty in the calibration of the heart rate technique rather than from the estimation of the correlation between the two techniques (Figure 2.2).

Table 2.2. Regression lines for the relationship between f_H derived $\dot{V}O_2$ and ODBA derived VO_2 along with R squared values for each behaviour based on the best model.

Parameters	Predictions	R squared %
Resting $\dot{V}O_2(f_H)$	(1.8708*resting $\dot{V}O_2$(ODBA))-81.0493	31.8
Diving $\dot{V}O_2(f_H)$	(0.1302*diving ODBA $\dot{V}O_2$(ODBA))+69.8013	0.038
Flying $\dot{V}O_2(f_H)$	(0.9842*flying ODBA $\dot{V}O_2$(ODBA))-3.0607	21.3

Table 2.3. Model terms and the corresponding AIC values for models predicting f_H derived $\dot{V}O_2$ from ODBA.

Model	Δ AIC	k
Behaviour*ODBA	0	8
Behaviour + ODBA	81.28	6
ODBA	215.86	4
Behaviour	460.16	5

Table 2.4. Predictive equations for estimating $\dot{V}O_2$ from ODBA from GLMMs along with R squared values for behaviour specific models.

Parameters	Predictions	R squared %
Resting $\dot{V}O_2$	(172.68*resting ODBA) + 16.133	31.8
Diving $\dot{V}O2$	(12.02*diving ODBA) + 76.588	0.038
Flying $\dot{V}O2$	(90.84*flying ODBA) + 48.218	21.3

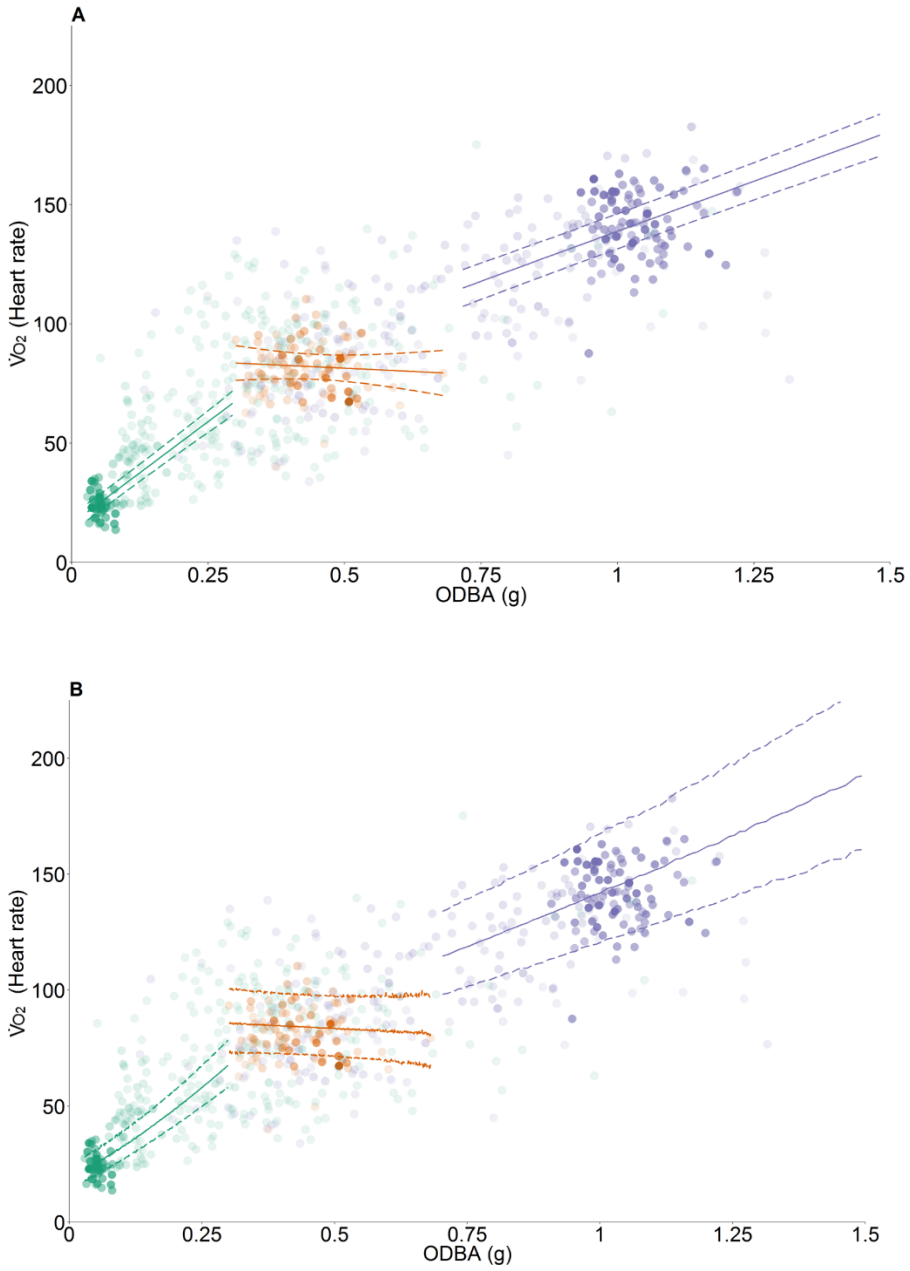

Figure 2.2. The relationship between overall dynamic body acceleration (g) and f_H derived energy expenditure (mil min^{-1}) Behaviour specific regression relationships (solid line) and 95% confidence intervals (dashed lines) for each behaviour (resting in green, diving in orange and flying in purple) are shown. Point transparency varies with duration of time spent in each behavioural bout. A. 95% confidence intervals are taken from the model estimates without taking into account the residual error associated with converting f_H to $\dot{V}o_2$ estimates. B. 95% confidence intervals from the bootstrapping method accounting for the residual error associated with converting f_H to $\dot{V}o_2$ estimates.

Discussion

Relatively few studies have investigated whether ODBA represents a robust proxy for energy expenditure across natural behaviours at high resolution in free-ranging birds (Duriez et al., 2014; Weimerskirch et al., 2016). Here we compared energy expenditure estimates across a range of natural behaviours in a free-living organism using both the established heart rate method and accelerometry. Across behaviours we find a good relationship between ODBA and $\dot{V}o_2$. Within individual behaviours we suggest that ODBA is a useable proxy of energy expenditure during flying and resting, thus opening up potential new avenues of research for quantifying energy budgets for individuals across key behaviours. However, some caution is necessary: we found that ODBA is less reliable at estimating energy expenditure during diving behaviour though this may be due in part to lower variation in ODBA during ODBA than within flight or resting. We combine these findings to provide usable behaviour specific calibration relationships between ODBA and $\dot{V}o_2$ to more accurately estimate energy expenditure using the accelerometry technique alone.

Comparison of oxygen consumption estimates

Whilst there was a good relationship between the estimates made with both approaches, and ODBA estimates of $\dot{V}o_2$ for flight and diving sit well on the line of equality, ODBA overestimates f_H-derived $\dot{V}o_2$ for resting behaviour. It is known that ODBA estimates of energy expenditure during inactivity tend to be poorer than in high activity due to movement making up a small proportion of energy expenditure during inactivity (Green et al., 2009; Weimerskirch et al., 2016). Differences in estimates across the two techniques for resting may also have arisen because the underlying laboratory based calibrations with VO_2 that underpin our estimates were undertaken in different conditions. Although both ODBA derived $\dot{V}o_2$ and f_H derived $\dot{V}o_2$ lab calibrations for great cormorants (*Phalacrocorax carbo*) were based on the same captive individuals, they were conducted in different seasons (November and March/June respectively) (Gómez-Laich et al., 2011; White et al., 2011; Wilson et al., 2006a). Seasonal variation in BMR is well documented (Smit and McKechnie, 2010). In this case

the cormorants had lower BMR in the summer months (C.R. White, P.J. Butler, G.P. Martin, unpublished data). The higher resting VO_2 values estimated by ODBA compared to f_H may be due to the higher resting metabolic rate incorporated into the ODBA calibration. Thus since ODBA is not sensitive to changes in BMR and cannot record seasonal variation in metabolic rate, this may be a limitation to this approach in studies trying to estimate seasonal changes in energy expenditure within a population or species. A strength of the approach described here is that since the $f_H/\dot{V}o_2$ calibrations were made during the summer, our new predictive equations allow $\dot{V}o_2$ to be estimated from ODBA during the summer months, thus accounting for seasonal changes in BMR.

Estimates of flight costs are lower than expected based on body mass alone (Bishop et al., 2002) but consistent with previous estimates based on calibrations from a congeneric species the great cormorant (White et al., 2011). It is possible that both f_H and ODBA underestimate $\dot{V}o_2$ during flight since Ward et al., (2002) show that estimates of $\dot{V}o_2$ during flight in two species of geese would be underestimated based on f_H during flight, and a walking-only calibration relationship. This is due to differences in calibration relationships for walking and flying in these species of geese. However in great cormorants the original calibration line between f_H and $\dot{V}o_2$ intersects with modelled estimates of flight $\dot{V}o_2$ suggesting the f_H- $\dot{V}o_2$relationship is robust for flight (Bishop et al., 2002; White et al., 2011). Additionally, the close agreement of both our ODBA and f_H-derived estimates of $\dot{V}o_2$ during flight suggest that ODBA based estimates are also accurate. This is either a coincidence, or provides support for the previous papers and methodologies. However, more research on the true costs of flight in unrestrained birds under natural conditions is urgently needed (Elliott, 2016).

ODBA as a predictor of energy expenditure

We found ODBA to be a good predictor of $\dot{V}o_2$; our best overall model, which includes the effect of behaviour, is comparable to other studies and calibrations suggesting there is considerable value in this method when used across a range of behaviours. The R^2 for our overall model is comparable but slightly lower than

studies comparing partial dynamic body acceleration and energy expenditure by doubly labelled water in the wild (R^2 = 0.73 in thick billed murres (Elliott et al., 2013a) and R^2 = 0.91 in pelagic cormorants (Stothart et al., 2016)) and consistently lower than measurements obtained on treadmills in the laboratory (R^2 = 0.81- 0.93 for four bird and mammal species (Halsey et al., 2009)) and experimental dive tanks (R^2 = 0.83 for green turtles (Enstipp et al., 2011). The R^2 value from this study is expected to be lower than those from previous studies as ODBA values are not daily averages as in most previous studies, but instead calculated over shorter time scales of behavioural bouts (Elliott, 2016; Green, 2011). However when our data are re-examined over a daily scale the R^2 the best overall model (marginal R^2 = 0.97) was higher than our calibration at finer temporal scale and more similar to previous calibrations using daily averages (See supplementary materials Figure S2.2).

Behavioural differences

The high temporal resolution of this study's calibration compared to previous studies (Elliott et al., 2013a; Jeanniard-du-Dot et al., 2016; Stothart et al., 2016) allows the more complex differences in energy expenditure between behaviours and resultant differences in predictive estimation equations between different behaviours to be quantified. All three behavioural modes had different predictive equations when estimating $\dot{V}o_2$ from ODBA. Similarly Elliott et al. (2013) and Stothart et al. (2016) found in calibrations of daily energy expenditure using the doubly labelled water method in the field, the most parsimonious models included classification of one behaviour separately from the others. What differs in our study however is the best model includes all behaviours separately. This may be driven by how well ODBA is able to reflect metabolic costs of movement in different media. ODBA provided reasonable estimates of $\dot{V}o_2$ in flight which is not unexpected given ODBA has been shown to correlate with heart rate in previous studies (frigate birds (Weimerskirch et al., 2016) and griffons (Duriez et al., 2014)). This is further supported by correlates between wing beat frequency and heart rate in bar headed geese (Bishop et al., 2015) which have a similar flapping flight to European shags. There is also evidence

from studies that one calibration of energy expenditure can be applied to all behavioural modes, though these studies did not involve diving or flying behaviour (Green et al., 2009; Wilson et al., 2006a).

ODBA provided poorer estimates of $\dot{V}o_2$ during diving which supports the finding of Halsey et al. (2011) that ODBA did not correlate with oxygen consumption over diving bouts in double crested cormorants in dive tank experiments (Halsey et al., 2011b). Cormorant species have partially wettable plumage (Grémillet et al., 2005) which causes high rates of heat loss and therefore high dive costs (Enstipp et al., 2005). As a result they may be susceptible to changes in metabolic rate within diving bouts (Enstipp et al., 2006; Grémillet, 1998) which would be expressed in changes in f_H but not in in ODBA, producing no clear relationship between ODBA and $\dot{V}o_2$.

Application of findings

By incorporating the error associated with the f_H derived $\dot{V}o_2$ calibration (White et al., 2011) we were able to derive relationships for each behaviour to predict oxygen consumption and its associated error from ODBA values. It is notable that it is the error originating from the laboratory based calibration between f_H and $\dot{V}o_2$ that is driving the large error distribution overall rather than the comparison between f_H and ODBA in the field. As the ODBA technique for measuring energy expenditure is becoming increasingly popular in the field, and provides fine scale information on the behaviour of the animal, it is essential to be able to use behaviour specific equations as this currently accounts for most of the uncertainty in free-living animal energy budgets (Collins et al., 2016; Wilson et al., 2006a). Our validation exercise indicates that for an average day our approach gives broadly similar estimates of energy expenditure to those derived from first principles and the literature (Supplementary materials Figure S2.4.). The behavioural-bout resolution of our calibration provides a natural range of behavioural bouts of varying lengths, created with free-ranging birds and natural behavioural bouts, meaning this calibration can be used at any temporal scale for resting and flight behaviour. While it is not possible to present a single equation that captures both elements of the residual error associated

with predictions, we provide a script that calculates estimates with SEE for a given value of ODBA (see supplementary materials).

This study therefore outlines an approach to generate behaviour-specific estimates of energy expenditure from ODBA, which can be used to more accurately to estimate total energy expenditure in the complex behaviour of free-living cormorant species. However the poor predictive power of ODBA during diving reinforces the idea that further temporal considerations may need to be incorporated for this behaviour. Whilst future recommendations include the simultaneous measurement of heart rate, acceleration and VO_2 with respirometry, we have provided equations that combine both heart rate and ODBA techniques as predictors of behaviour specific energy expenditure. ODBA derived behaviour-specific estimates of energy expenditure can help pave the way for future work answering ecologically important questions and understanding the fine scale costs of movement and foraging of diving seabirds.

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Appendix

Existing Calibration equations used in analyses
Calibration equation from Wilson et al., (2006) for the relationship between

ODBA and $\dot{V}O_2$
VO2 = (92.3*ODBA) + 52.1
Calibration equation from White et al., (2011) for the relationship between

heart rate and $\dot{V}O_2$.

$\dot{V}O_2$ = 0.0064*(HeartRate^1.63)*(mass^1.1)

Chapter 3

The energetic cost of parasitism in a wild population

The energetic cost of parasitism in a wild population

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Authors' contributions

OH, SB, FD and JG designed the study. OH, SB, FD, MN and MI collected the data. MI and KS provided essential data loggers for this study. OH processed the data, conducted the statistical analyses and wrote the manuscript. AB provided statistical advice. All authors (OH, SB, FD, MN, AB, MI, KS and JG) contributed to interpreting results and improvement of this paper.

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Abstract

Parasites have profound fitness effects on their hosts, yet these are often sub-lethal, making them difficult to understand and quantify. A principal sub-lethal mechanism that reduces fitness is parasite-induced increase in energetic costs of specific behaviours, potentially resulting in changes to time and energy budgets. However, quantifying the influence of parasites on these costs has not been undertaken in free-living animals. We used accelerometers to estimate energy expenditure on flying, diving and resting, in relation to a natural gradient of endo-parasite loads in a wild population of European shags *Phalacrocorax aristotelis*. We found that flight costs were 10% higher in adult females with higher parasite loads and these individuals spent 44% less time flying than females with lower parasite loads. There was no evidence for an effect of parasite load on Daily Energy Expenditure, suggesting the existence of an energy ceiling with the increase in cost of flight compensated for by a reduction in flight duration. These behaviour specific costs of parasitism will have knock-on effects on reproductive success, if constraints on foraging behaviour detrimentally affect provisioning of young. The findings emphasise the importance of natural parasite loads in shaping the ecology and life-history of their hosts, which can have significant population level consequences.

Introduction

Parasites are among the most successful life forms and infect nearly every known animal taxon (Dobson et al., 2008). It is well known that parasites can have major detrimental fitness consequences on their hosts. e.g. (Booth et al., 1993; Gooderham and Schulte-Hostedde, 2011; Reed et al., 2008b). Such consequences may arise due to multiple costs imposed by parasites, such as immune or stress responses or the direct extraction of resources from their hosts (Martin et al., 2003; Sheldon and Verhulst, 1996; Smyth and Drea, 2016). These costs are frequently sub-lethal, whereby they alter fitness-related traits, yet these processes are poorly understood because they are challenging to quantify (McElroy and de Buron, 2014). A principal sub-lethal effect of parasites that can readily be quantified is the impact on performance in terms of movement and foraging. This is a critical mechanism since impairment of these behaviours can have substantial negative fitness consequences (Binning et al., 2017; Lewis et al., 2009).

Since energy in wild animals is limited (Hammond and Diamond, 1997) there can be a trade-off between allocation of resources to parasite-induced immune responses and performance-related behaviours (Norris and Evans, 2000). Using an energetics framework with energy as the central currency can therefore shape our understanding of how parasites impose behavioural costs on their hosts, and whether hosts compensate for these costs by changing the duration of behaviours to regulate overall energy expenditure. An energetics framework requires the measurement of variation in performance-related behaviours in relation to parasite burdens (Sandland and Minchella, 2003). However, linking parasitism mechanistically to performance in free-ranging animals has been undertaken in only a very small number of cases and often in tractable invertebrate systems (Sandland and Minchella, 2003).

For most taxa, foraging for resources is tightly linked to fitness (Binning et al., 2017; Daunt et al., 2007), and can be quantified through its impacts on rates of energy expenditure and gain (Elliott et al., 2014b; Tomlinson et al., 2014). Foraging is an energetically costly behaviour, especially for breeding individuals commuting between a fixed breeding site and foraging locations, because the

energetic costs of travel imposes limits on the allocation of time and resources for self-maintenance and offspring provisioning (Burke and Montevecchi, 2009; Dosselli et al., 2016). Even with unlimited energy availability, energy expenditure for foraging and other activities is limited in wild animals (Hammond and Diamond, 1997). There is evidence that intrinsic constraints may impose an 'energy ceiling' in terms of a limit or optimum to Daily Energy Expenditure (DEE, the total energy expenditure of an animal over 24 h) defined as the sum of the cost of activities multiplied by time spent on those activities (Speakman, 1997). As such, to maintain DEE, any increase in costs of a behaviour may be compensated for by reducing the time allocation to that behaviour. Such compensation may then lead to changes in fitness (Biro and Stamps, 2010; Mathot and Dingemanse, 2015). However, these processes have not previously been quantified in free-living animals.

Here, we quantify the costs of nematode endo-parasites on the cost and time allocation of specific behaviours (flying, resting and diving) in breeding European shags *Phalacrocorax aristotelis*. Breeding adult shags are central place foragers commuting between the nest site and foraging grounds, experiencing high energetic costs of foraging activities (Enstipp et al., 2005). Furthermore, recent experimental work has illustrated the critical role of parasitism in European shag reproduction (Granroth-Wilding et al., 2014; Reed et al., 2008b). As such, they are a useful study species to test the effects of parasitism on the cost and allocation to key foraging activities. In this study, we aim to quantify the costs of endo-parasites by testing three main hypotheses: H1) Parasite load is linked to an increase in the energetic cost of behaviours; H2) Time allocated to affected behaviours is negatively related to parasite load; H3) Increases in energetic cost of behaviours will be compensated for by changes in time allocation resulting in no link between parasite load and Daily Energy Expenditure.

Materials and Methods

Study site and species

The study was carried out on the Isle of May National Nature Reserve, south-east Scotland (56°11'N, 2°33'W) during the breeding seasons of 2014-2017. All individuals were part of a long-term population study and are marked with a unique metal ring and a darvic ring for identification. Adults are sexually dimorphic, with males 22% larger than females (Wanless and Harris, 1997), and are sexed by vocalisations (Snow, 1960). Populations of European shags *Phalacrocorax aristotelis* are susceptible to nematode gastro-intestinal parasites, in particular anisakid nematodes *Contracaecum rudolphii*. Previous sampling of this population through dissection, faecal egg counts and endoscopy has shown a high prevalence of *C. rudolphii* (Burthe et al., 2013; Granroth-Wilding et al., 2014; Reed et al., 2008b), though parasite loads vary markedly between individuals (Burthe et al., 2013; Granroth-Wilding et al., 2014). Effects are usually sub-lethal, whereby parasites compete with the host for nutrients and initiate costly immune responses (Abollo et al., 2001). Shags become infected with third stage larvae via their fish diet. Larval worms moult to become sexually mature adults which attach to the lining of the proventriculus and lower oesophagus in the final seabird host (Abollo et al., 2001; Burthe et al., 2013).

Measuring parasite loads

Adult European shags were captured on the nest using a crook on the end of a long pole. Endoscopy to quantify individual parasite burdens was undertaken in early chick rearing (when the chicks were between 5 and 38 days old). To ensure that individuals had empty stomachs, endoscopy was performed between 03:30 and 07:30, before they had left for their first foraging trip of the day. Worm burdens were counted visually using the endoscope video screen, though counts of worms for burdens of >40 were not possible due to the number of worms preventing good visibility. Counts of worms greater than this were recorded as > 40. These methods were found to be repeatable within an individual across a season (Burthe et al., 2013). For detailed endoscopy

methods see Burthe et al. (2013). All endoscopy was performed by trained personnel (S. Burthe) holding a personal licence operating under a project licence issued by the UK Home Office under the Animals (Scientific Procedures) Act 1986.

Measuring energy expenditure

All birds were then equipped with tri-axial accelerometers (D3GT, little Leonardo, Tokyo, Japan, AXY3-Depth, Technosmart, Rome, Italy and Gulf Coast Data Concepts X8) to measure the energetic cost of variation in parasite load. Accelerometers ranged in mass from 6.5-9g but all were <0.7% of the minimum shag body weight in this study well within the recommended acceptable limit of logger weights. Accelerometers were set to record at 25 or 50 Hz and attached on the midline of the mid back of individuals (as close to the centre of gravity as possible) using Tesa tape. All birds were successfully recaptured and accelerometers were retrieved after four days of deployment (92 deployments on endoscoped individuals across four years with 57 unique individuals; n=4 in 2014, n=24 in 2015, n=39 in 2016, n=25 in 2017).

Data logger traces were used to differentiate between diving, flying and resting (the three main activities of shags) in two steps. Firstly the Ethographer application, which was developed to classify behaviour states in European shags, (Sakamoto et al., 2009) in IGOR Pro software (Wavemetrics Inc., Portland, OR, USA, 2000, version 6.3.5) was used to assign data as diving (including surface and subsurface periods) or non-diving behaviour, through supervised cluster analysis using k means methods on the depth trace (Sakamoto et al., 2009). In the case of the accelerometers where depth data were not available, cluster analysis was performed on the surge axis. Secondly, the remaining accelerometer data was assigned as either flight or resting behaviour (either at sea or on land) using frequency histograms of the standard deviation of the heave axis and pitch (calculated over 60 seconds) (Collins et al., 2015b).

Overall dynamic body acceleration (ODBA) was calculated by first smoothing each of the three acceleration channels with a running mean to represent

acceleration primarily due to gravity. In our study, the running mean was 1s (i.e. 25 data points for 25Hz accelerometers) as in (Collins et al., 2015b). The smoothed value was then subtracted from the corresponding unsmoothed data for that time interval to produce a value of g resulting primarily from dynamic acceleration (Wilson et al., 2006a). Derived values were then converted into absolute positive units, and the values from all three axes were summed to give an overall value for dynamic acceleration experienced.

Estimates of oxygen consumption ($\dot{V}o_2$ml/min) were derived from ODBA values using calibration equations from Hicks et al. (2017) to determine behavioural specific energy expenditure values for all behavioural bouts. For each individual, we calculated a mean daily rate of oxygen consumption for each behaviour, averaging all bouts of that behaviour on that day. Daily energy expenditure (DEE) was the sum of the energetic costs of all behavioural bouts within a full 24-hour period of activity. Individuals had between one and four of these periods, dependent on the length of the logger deployment. We treated estimates of energy expenditure at the individual level as a measurement, as in previous studies of field energetics using a variety of approaches (Elliott et al., 2013c; Sala et al., 2014).

Extrinsic variables

We incorporated extrinsic variables that impact on the foraging behaviour of breeding adults since the effect of parasitism in this system varies across environmental conditions (Granroth-Wilding et al., 2014). Mean population productivity (measured as the average number of fledged young per incubated nest in a series of long-term monitoring plots from the wider island population) was included as a measure of annual environmental conditions, following (Granroth-Wilding et al., 2014). Chick age of the oldest chick in the brood was estimated from wing length at ringing at ~20 days of age (a reliable indicator of chick age (Reed et al., 2012)) and used to back calculate an estimate of age in days at time of logger deployment. Brood size (number of chicks at logger deployment) was also incorporate in analyses, as adult energy expenditure and

foraging effort is likely to vary with the total brood mass that must be provisioned (Grémillet, 1997).

Statistical Analysis

To test H1, we first considered the cost of three behaviours in response to parasite load. We modelled mean daily rate of oxygen consumption for each bird each day ($\dot{V}o_2$ml/min) separately for flight, diving and resting behaviours using linear mixed effects models (LMMs). Parasite load, mean population productivity, brood size and chick age in days were fitted as continuous explanatory variables and we accounted for variation among individuals and years by including individual, year and a year by individual interaction as random factors. Interactions between parasite load and each of the other three explanatory variables were considered. We fitted models for males and females separately due to non-independence of nest pairs and differing parasite load distributions between sexes and indications that similar parasite loads have different impacts on males and females (Granroth-Wilding et al., 2014; Reed et al., 2008b). See ST1 for a description of model structures and explanations; all possible subsets of fixed effects are considered when running the model selection (subject to the standard restriction that interaction terms are only included alongside the corresponding main effect terms). To test H2 and quantify how the proportion of time spent per day in behaviours changed with parasite load, we modelled logit transformed proportion per day of each behaviour (flight, diving and resting on land) in separate models, using the same set of explanatory variables as in the analysis of costs of behaviours. Finally, to test H3 we modelled Daily Energy Expenditure using the same model structure as those for the individual behaviours.

In all model sets, model selection was based on Akaike's information criterion (AIC), which penalises the inclusion of parameters in models, and hence should lead to the removal of unnecessary parameters (Burnham and Anderson, 2001). The model with the lowest AIC is usually chosen to be the 'best' model, but models within two Δ AIC of the lowest value are generally considered to have

similar empirical support to that of the best model. All models were fitted using the lme4 package in R (Bates et al., 2014; R Core Team, 2015)

Finally, to investigate the links between energetic costs, behaviours and DEE in full, we used predicted values from our models to estimate the total cost of each behaviour per day in high and low parasite burden scenarios. This was achieved by multiplying the predicted energetic rates by the predicted proportion of time spent in each behaviour under maximum and minimum parasite loads measured in the study. We also summed the total simulated costs of each of the three behaviours to estimate DEE for each parasite burden scenario. This enables us to understand whether changes in costs of behaviours were compensated for via changes in duration of that behaviour.

Results

The effect of parasite load on the energetic cost of behaviours

In females, an effect of parasite load on the cost of foraging behaviours was detected in all three behaviour specific models; effect sizes varied with the largest effect apparent in flight behaviour (see Figure 3.1 for comparison of all behaviours on the same scale). In males, there was weak evidence for a positive effect of parasitism on flight behaviour but no evidence for the effect of parasitism on dive or rest behaviour.

Flight behaviour

The best supported model for the effect of parasitism on the cost of flight in females showed a positive relationship between parasite load, with the cost of flight behaviour increasing by ~10% from the minimum to maximum parasite load (see Table S3.2). This model also incorporated a positive effect of brood age and a negative effect of brood size on the cost of flight (see Figure S3.2). A positive effect of parasite load was included in all of the 6 best supported models (within 2 Δ AIC of the top model). For males, the best supported model included a positive effect of parasite load as well as a positive effect of brood age and an interaction between brood age and parasite load (see Figure S3.2). However, in this case, parasite load was only included in 4 of the 6 best

supported models and the null model is 1.53 Δ AIC within the top model, suggesting some caution in interpreting this result (see Table S3.2).

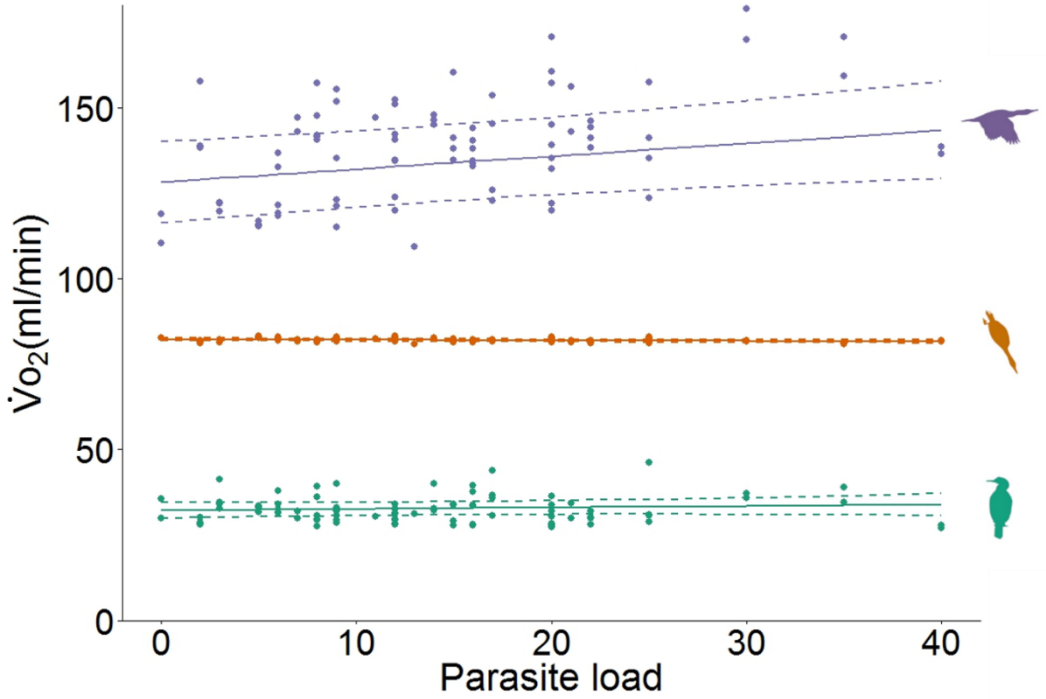

Figure 3.1. The effect of parasite load on the cost ($\dot{V}o_2$ ml min^{-1}) of flight, diving and resting behaviour for female European shags. Solid lines represent predicted values from the best supported model for each behaviour and dashed lines represent 95% confidence intervals. Points represent mean daily rate of oxygen consumption for each behaviour of each bird on each day. Purple is cost of flight, orange is cost of diving and green is cost of resting. All three behaviour predicted lines are extracted from separate behaviour specific models.

Dive behaviour

The best supported model for the effect of parasite load on the cost of diving behaviour for females included a negative effect of parasite load, though the effect size was small in that the dive costs at maximum observed parasite loads were just 0.7% lower than at the minimum observed parasite load. Equally some caution must be exercised as parasite load is only included in 2 of the 4 best supported models (see Table S3.3). For males, the best model was the null model.

Rest behaviour

The best supported model for females included a positive effect of parasitism, with the cost of rest increasing by 5% from the minimum to maximum parasite load. This model also incorporated a negative effect of brood size, a positive effect of brood age (see Figure S3.3) and mean population productivity as well

as an interaction between parasitism and mean population productivity (Table S3.4). In lower productivity years there is a greater positive relationship between parasite load and the cost of rest than in high productivity years. However, the null model is only 1.16 Δ AIC within the top model. The best supported model for males was the null model.

The effect of parasite load on the proportion of time spent in foraging behaviours

In females, the effect of parasite load was found to be strongly negatively related to the proportion of time spent in flight behaviour but no effect on diving or resting time budgets could be identified (see Figure 3.2). In males, there was no evidence for the effect of parasite load on the time budgets of any behaviour.

Proportion of time spent in Flight

The best supported model showed a negative effect of parasite load on the proportion of time spent in flight for females (Table S3.5) such that individuals with the highest parasite loads spend 44% less time in flight than individuals with the lowest parasite loads (see Figure 3.2.). The best supported model for males (ST5) did not include parasite load but contained a positive effect of brood age on the proportion of time spent in flight behaviour (see Figure S3.4).

Proportion of time spent in diving

The best supported model for females included a positive effect of brood age on the proportion of time spent diving (Table S3.6). The best supported model for males also included a positive effect of brood age on the proportion of time spent in diving behaviour (see Figure S3.5).

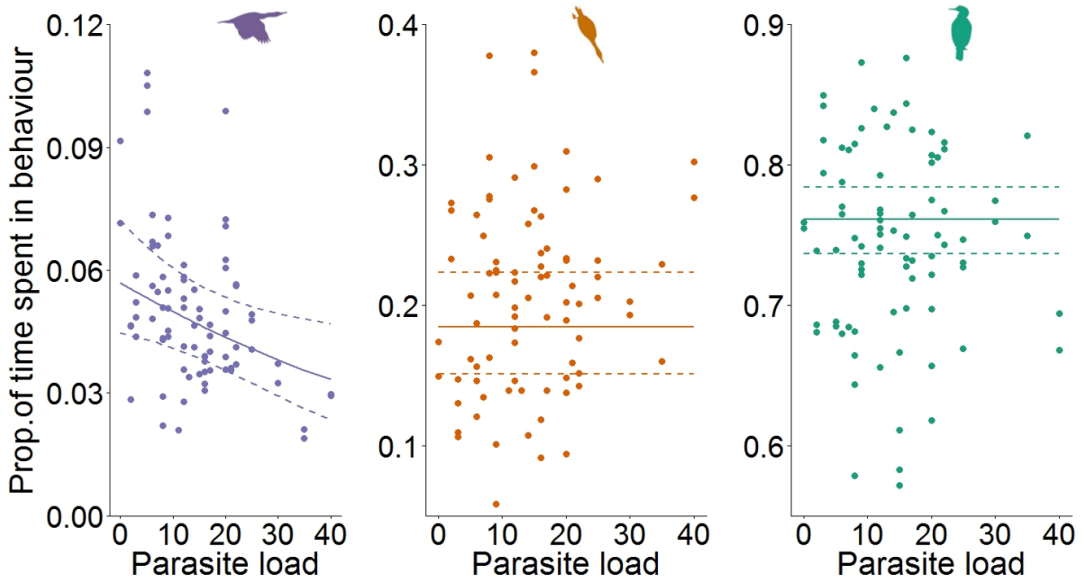

Figure 3. 2. Impact of variation in individual parasite load on the proportion of time spent in flight, diving and rest with respect to other foraging behaviours in female European shags. Solid line represents predicted values from the best supported model for females dashed lines represent 95% confidence intervals. Points represent the proportion of time spent in each behaviour for each bird each day. Different panels represent the three behaviours left to right; flight, diving and rest. Models are fitted separately for each behaviour therefore predicted proportions do not sum to one.

Proportion of time spent in resting

The best supported model for females included a negative effect of brood age on the proportion of time spent resting. For males, the best supported model also included a negative effect of brood age on the proportion of time spent resting (Table S3.7).

The effect of parasite load on Daily Energy expenditure

There was no evidence for an effect of parasite load on Daily Energy Expenditure for females or males (Table S3.8). For females, the model containing parasite load was 2.36 AIC units greater than the best-supported model and for males the model containing parasite load was 2.24 AIC units greater than the best-supported model. The best supported models showed only a positive effect of brood age on DEE for both males and females (see Figure S3.7.).

Predicted behavioural costs

Despite costs of flight increasing with parasite load, the maximum parasite load scenario has a lower total energy expenditure spent in flight. Given that the

proportion of flight per day decreases with parasite load this suggests that individuals with high parasite loads reduce flight time more than is actually required (see Figure 3.3).

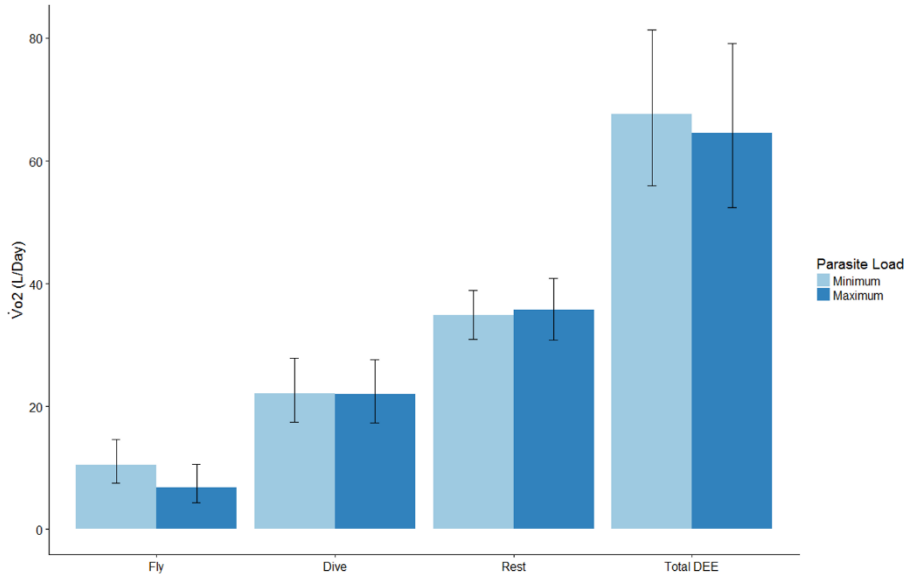

Figure 3.3. Predicted total energy expenditure ($\dot{V}O_2$ L Day^{-1}) for female shags in each behaviour per day with 95% confidence intervals. Total energy expenditure values for each behaviour are calculated based on predicted values for the proportion of time spent in each behaviour multiplied by the predicted rate of energy expenditure for that behaviour. Total DEE is calculated by summing the total energy expenditure for the three behaviours. Light blue bars represent predictions based on a minimum natural parasite load scenario and dark blue bars represent predictions based on maximum parasite loads. If behavioural costs and time budgets balance out there should be no difference in total behavioural energy expenditure between maximum and minimum parasite load scenarios.

Discussion

In this study, we quantified the energetic cost of parasitism to individuals of a free-living population for the first time. We detected a change in the energetic cost of behaviours and time budgets due to variation in natural endo-parasite load in female European shags but no relationship between parasite load and Daily Energy Expenditure in either sex. Specifically, in support of H1 and H2, we found the cost of flight to be higher in females with higher parasite loads and these individuals spent a smaller proportion of their time in this behaviour than females with lower parasite loads. We found a small effect of parasitism on the cost of diving and resting behaviour in female shags but no change to their time energy budgets as a result of parasite load. Overall, in support of H3, this compensation suggests that European shags are limited in their Daily Energy

Expenditure and must compensate for increased activity costs by reducing their duration this suggests energetic trade-offs exist between performance-related behaviours and immune responses to parasitism.

Flight usually comprises a large proportion of an individual's Daily Energy Budget in volant birds with flapping flight (Elliott, 2016). For species such as the European shag, where energy expenditure is limited by a tight energy budget or optimal energy ceiling, we would expect a reduction in investment in more costly behaviours. Accordingly, we found that individuals with higher parasite burdens and greater flight costs spent a smaller proportion of time in flight than individuals with low parasite burdens. Similarly, experimentally parasitized honey bees were found to perform a lower number of daily flights than control individuals (Alaux et al., 2014).

While flight behaviour in any species requires large energy outputs and efficient muscle use, endo-parasites can affect nutrient assimilation and muscle efficiency essential for flight (Colditz, 2008). Shags like other cormorant species are continuous flapping flyers and have limited flight performance as a trade-off to their diving ability (Watanabe et al., 2011). Consequently, any damage to muscle efficiency or feather quality could have significant impacts on flight costs. There is evidence of the negative impacts of endo-parasites on feather repair and development as well as stress on feather quality in other species of birds (Moreno-Rueda, 2015; Pap et al., 2013). Additionally preen gland size is negatively related to immune function which, via preen oil production, can also impact feather condition function (Moreno-Rueda, 2015; Pap et al., 2013).

By measuring the costs of active behaviours such as flight and diving behaviour, we can gain an understanding of how these high cost behaviours might link to energetic limits. However, resting behaviour costs should partially reflect the maintenance costs of an individual and be elevated with an increase in immune response (Careau et al., 2012; Ots et al., 2001). That we see a small increase in the cost of resting behaviour with increased parasite load could be an indication of increased maintenance cost. However it is known that accelerometry is not a good proxy for resting costs in active animals (Green et al., 2009) and further

measurements of Resting or Basal metabolic rate using a more appropriate approach is recommended.

In establishing the response of DEE to parasite load our results suggest that adult shags may have an optimum energy ceiling, as demonstrated in several other seabird species during the breeding season (Mathot and Dingemanse, 2015; Welcker et al., 2010). This optimum energy ceiling means that any increase in cost to an activity must be counteracted by either a reduction in duration of that activity or a reduction in cost of other activities. This assertion is supported by our finding that individuals with more costly flight behaviour spent a lower proportion of their time per day in flight, ensuring that DEE was unaffected. In calculating total behavioural costs (Figure 3.3) we found individuals with high parasite loads decreased the proportion of time spent in flight more than would be predicted solely from the increase in the cost of this behaviour as there was an overall decrease in the mean total cost of flight per day (see Figure 3.3). This trade-off between flight costs and allocation suggests that changes in the proportion of time spent in flight may also be compensating for other costs or changes in energy use. We would expect that any increase in maintenance costs arising from an immune response, that were not measureable in this study, would require additional compensation through reduced activity (Mathot and Dingemanse, 2015). As flight is the most costly behaviour that shags can use to divest energy expenditure, it may be that maintenance costs in the highly parasitized individuals are being compensated for with the extra reduction in flight time.

Previous work has shown that extrinsic variables such as wind, presence of food in the stomach and other environmental conditions can affect foraging energetics, behaviour and breeding success (Lewis et al., 2015; Sato et al., 2008). Our findings are consistent with this finding, providing additional evidence that environmental drivers are important in energy use. Our finding that brood age was positively related to flight costs in females is expected, since adults provisioning large chicks return from foraging grounds with larger food loads, which incurs higher costs on the inbound flight (Sato et al., 2008). This relates to the experimental evidence of Reed et al., (2008) where females spent

more time foraging with increasing age of their offspring when they were relieved of their parasite load. Similarly, DEE also increased with brood age which we interpret to mean that shags have an optimal energy ceiling at any given stage of the breeding season, perhaps reflecting the demands of provisioning the chicks and/or investment made to that point. DEE of provisioning birds often increases to accommodate extra energetic needs of offspring (Welcker et al., 2015), however the scope to which DEE can be raised further in response to parasites appears to be limited, either due to physiological, extrinsic or other factors(Elliott et al., 2014b)

The negative relationship found between brood size and cost of flight behaviour may relate to the quality of the individuals, such that individuals with larger broods are likely to be higher quality individuals in better condition with more energy to assign to maintenance such as feather condition and therefore may experience lower costs in flight. It is known that parasite effects can vary with environmental conditions (Granroth-Wilding et al., 2014) therefore it is important to note that effects in this study were all from data collected in years with high mean population productivity (our proxy for environmental conditions).We would expect to see more extreme effects of parasitism in lower productivity years where poor foraging conditions can cause individuals to be under more severe energetic constraint.

We consistently found limited evidence for the effect of parasite load on energy budgets or costs of behaviours in male European shags. This difference among sexes corroborates previously established differences in investment in reproduction and consequently different constraints on energy expenditure in females compared to males across many species (Harms et al., 2014). Experimental work in shags also shows stronger effects on foraging time in females than males when parasites are removed (Reed et al., 2008b). The larger of the sexes often show higher foraging efficiency in bird species (Lewis et al., 2015). Consistent with the assumption that females (the smaller sex in shags) are more constrained energetically, only females showed an energetic and behavioural response to parasitism despite both sexes showing a positive increase in DEE with brood age. These sex differences in the impact of parasites

could have consequences for survival; indeed, previous work has shown that during winter female shags have lower survival than males (Brooks et al., 2002).

Conclusions

In most parasite-host systems there is marked heterogeneity with respect to parasite load within the population, which often leads to demographic differences among individuals (Granroth-Wilding et al., 2016). In this study, we demonstrated that, parasite load was related to energy expenditure and time budgets of foraging behaviours, which may be a key process underpinning the demographic consequences of parasites. This work demonstrates that energetics is a powerful framework to aid the understanding of individual-level mechanisms driving life-history. This study provides a potential mechanism behind experimental evidence of sex biased fitness effects of parasitism in a free-ranging population. The findings emphasise the importance of natural parasite loads in shaping the ecology and life-history of their hosts, which can have significant population level consequences (McElroy and de Buron, 2014).

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Supplementary materials

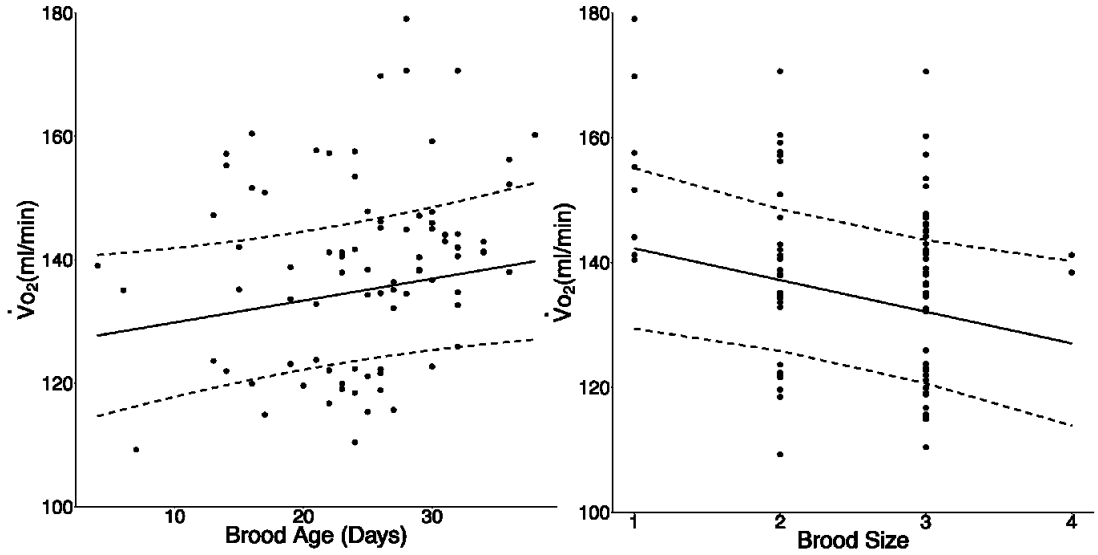


Figure S3.1.Impact of variation in brood size and brood age (days) on the energetic cost ($\dot{V}o_2$ ml min^{-1}) of flight in females. Solid lines represent predicted values from the best supported model and dashed lines represent 95% confidence intervals. Other variables in the best supported model were fixed at their mean to look solely at the effect of the parameter of interest.

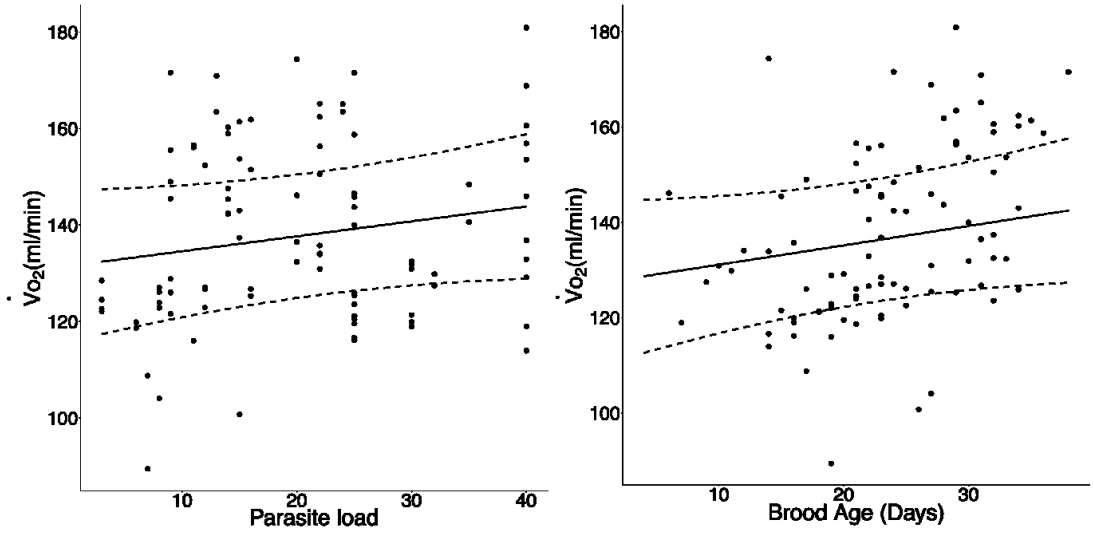


Figure S3.2.Impact of variation in parasite load and brood age on the energetic cost ($\dot{V}o_2$ ml min^{-1}) of flight in males. Solid lines represent predicted values from the best-supported model and dashed lines represent 95% confidence intervals. Other variables in the best supported model were fixed at their mean to look solely at the effect of the parameter of interest.

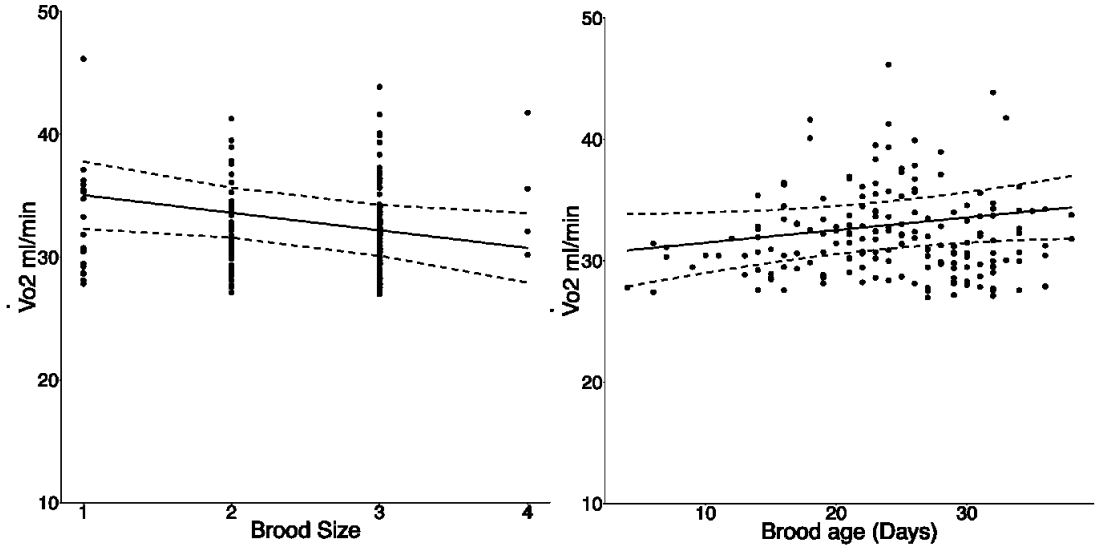


Figure S3.3. Impact of brood size and brood age on the energetic cost ($\dot{V}o_2$ ml/min) of rest in females. Solid lines represent predicted values from the best-supported model and dashed lines represent 95% confidence intervals.

Figure S3. 4. The effect of brood age (days) on the proportion of time spent flying in male shags. Solid lines represent predicted values for the best supported model and dashed lines represent 95% confidence intervals.

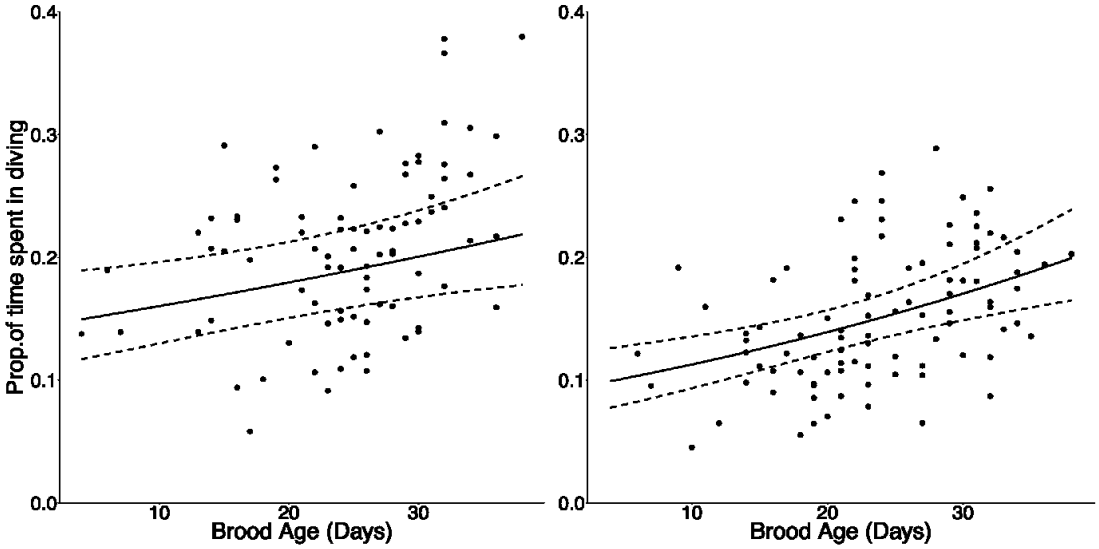


Figure S3.5.The effect of brood age (days) on the proportion of time spent diving in female and male shags (left to right). Solid lines represent predicted values for the best supported model and dashed lines represent 95% confidence intervals.

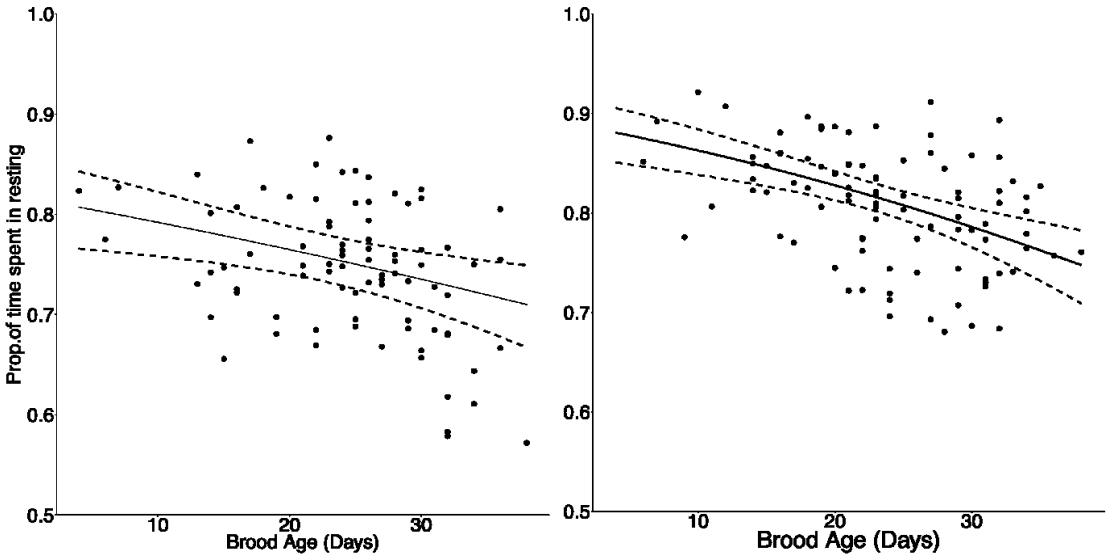


Figure S3.6.The effect of brood age (days) on the proportion of time spent resting in female and male shags (left to right). Solid lines represent predicted values for the best supported model and dashed lines represent 95% confidence intervals.

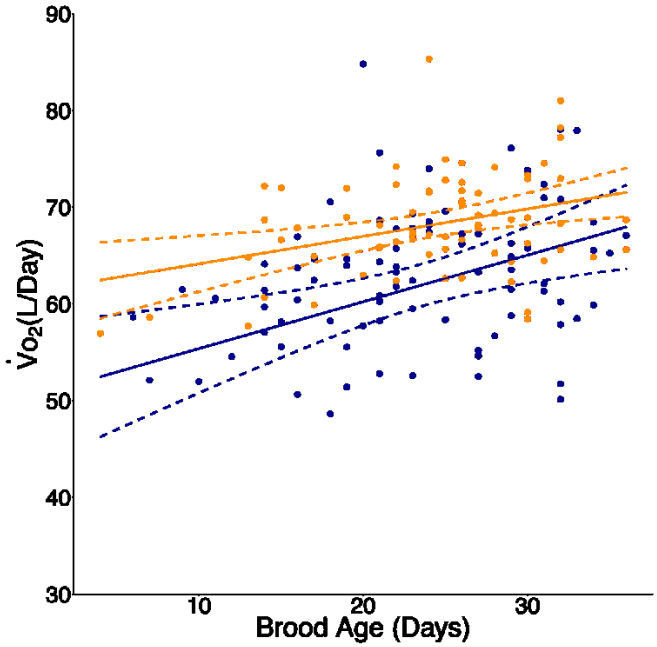


Figure S3.7. The effect of brood age on the Daily energy expenditure in adult shags. Solid lines represent predicted values for the best supported model and dashed lines represent 95% confidence intervals males in dark blue and females in orange.

Table S3.1. Model variables and structure for the three questions we ask in this study. Models were run separately for males and females on all occasions. Random effects are included in all models; the set of explanatory variables to include is selected by fitting all possible subsets of these variables, and comparing the resulting model fits using AIC.

Type		Variable
Response variables	Qu. 1: Does parasite load drive an increase in the energetic cost of foraging behaviours	Energetic cost of flight Energetic cost of diving Energetic cost of resting
	Qu. 2: Does parasite load drive changes in time budgets, specifically a reduction in the proportion of time spent in foraging behaviours	Proportion of time spent flying Proportion of time spent diving Proportion of time spent resting
	Qu. 3: Does Daily Energy Expenditure vary in relation to parasite load, or are any changes in costs of behaviours compensated for by a change in time budget of that behaviour.	Daily Energy Expenditure
Explanatory variables		Parasite load
		Brood Size
		Mean pop. Prod
		Brood Age
	Effects of parasites could vary with reproductive demand of the brood, it is also important to account for brood size as it is known to influence foraging behaviour	Parasite load * Brood Size
	Effects of parasites have been found to vary with prevailing environmental conditions	Parasite load * Mean pop. Prod
	Effects of parasites could vary with reproductive demand of the brood, it is also important to account for brood age as it is known to influence foraging behaviour.	Parasite load * Brood Age
Random structure		Individual
		Year
		Individual * Year

Table S3.2. The top ten best-supported models for the effect of parasite load on the cost of flying behaviour for male and female European shags

	(Int)	Parasite load	Brood Size	Mean pop. Prod	Brood Age	Parasite load * Brood Size	Parasite load * Brood Age	df	logLik	ΔAIC
Female	-0.20	0.23	-0.22		0.15			8	-66.83	0.0
	-0.29	0.22	-0.17					7	-68.39	0.66
	-0.30	0.21	-0.17			-0.14		8	-67.36	1.05
	-0.22	0.23	-0.21		0.12	-0.10		9	-66.28	1.41
	-0.26	0.25						6	-70.01	1.52
	-0.24	0.20	-0.20		0.16		0.12	9	-66.38	1.62
	-0.14	0.23	-0.23	0.20	0.14			9	-66.64	2.14
	-0.29	0.18	-0.19		0.13	-0.14	0.17	10	-65.42	2.29
	-0.29		-0.23		0.15			7	-69.25	2.39
	-0.19	0.26			0.10			7	-69.26	2.42
Male	-0.08	0.19			0.17		0.23	8	-86.42	0.00
	-0.09				0.21			6	-89.25	0.93
	-0.18	0.18			0.20			7	-88.35	1.46
	-0.16							5	-90.69	1.53
	-0.25	0.19						6	-89.63	1.69
	-0.16	0.18		-0.27	0.19		0.26	9	-86.13	1.87
	-0.08	0.19	-0.01		0.17		0.23	9	-86.41	2.44
	-0.15	0.15		-0.40	0.21	0.18	0.22	10	-85.43	2.98
	-0.13			-0.11	0.21			7	-89.21	3.19
	-0.09		-0.03		0.22			7	-89.22	3.21

Table S3.3. The top ten best-supported models for the effect of parasite load on the cost of diving behaviour for male and female European shags

	(Int)	Parasite load	Brood Size	Mean pop. Prod	Brood Age	Parasite load * Mean. Pop	df	logLik	ΔAIC
Female	0.15	-0.27					6	-106.96	0.00
	0.22						5	-108.44	0.64
	0.21	-0.27		0.31			7	-106.39	1.26
	0.30			0.34			6	-107.95	1.98
	0.14	-0.28	-0.06				7	-106.88	2.23
	0.16	-0.27			0.03		7	-106.93	2.33
	0.23				0.05		6	-108.37	2.83
	0.23	-0.27		0.27		-0.12	8	-105.99	2.90
	0.17		-0.10				6	-108.52	3.13
	0.22	-0.29	-0.10	0.36			8	-106.16	3.25
Male	-0.39						5	-95.19	0.00
	-0.34				0.17		6	-94.22	0.35
	-0.46	0.15					6	-94.59	1.10
	-0.30			0.22			6	-94.75	1.41
	-0.41	0.15			0.16		7	-93.67	1.57
	-0.28			0.16	0.15		7	-93.98	2.21
	-0.39		-0.01				6	-95.19	2.28
	-0.34		-0.06		0.19		7	-94.11	2.46
	-0.36	0.15		0.24			7	-94.21	2.65
	-0.46	0.15	0.00				7	-94.59	3.43

Table S3.4. The top ten best-supported models for the effect of parasite load on the cost of resting behaviour for male and female European shags

	(Int)	Parasite load	Brood Size	Mean pop. Prod	Brood Age	Parasite load * Brood Size	Parasite load * Mean pop.	df	logLik	Δ AIC
Female	0.19	0.12	-0.28	0.17	0.19		-0.37	10	-112.00	0.00
	0.04	0.05	-0.16			-0.38		8	-114.67	0.23
	0.16	0.10	-0.22	0.16			-0.40	9	-113.44	0.29
	0.16	0.12		0.05			-0.38	8	-114.73	0.35
	0.13	0.08	-0.21	0.15		-0.22	-0.30	10	-112.36	0.73
	0.07	0.08	-0.22		0.18	-0.33		9	-113.70	0.81
	0.07							5	-118.72	1.16
	0.16	0.10	-0.26	0.16	0.17	-0.18	-0.30	11	-111.31	1.28
	0.09		-0.26		0.24			7	-116.46	1.36
	0.18	0.14		0.04	0.14		-0.36	9	-114.00	1.40
Male	-0.08							5	-125.70	0.00
	-0.06				0.16			6	-125.01	0.90
	-0.07		0.10					6	-125.25	1.38
	-0.06	-0.08						6	-125.47	1.83
	-0.07			0.04				6	-125.66	2.21
	-0.06		0.06		0.13			7	-124.86	2.94
	-0.05	-0.05			0.15			7	-124.91	3.03
	-0.06			0.00	0.16			7	-125.01	3.24
	-0.05	-0.07	0.10					7	-125.04	3.30
	-0.07		0.10	0.01				7	-125.25	3.71

Table S3.5. The top ten best-supported models for the effect of parasite load on the proportion of time spent in flight per day for male and female European shags

	(Int)	Parasite load	Brood Size	Mean pop. Prod	Brood Age	Parasite load * Brood Size	Parasite load * Mean pop. Prod	df	logLik	ΔAIC
Female	0.18	-0.35						6	-89.78	0.00
	0.20	-0.32	0.16					7	-89.03	0.89
	0.22	-0.35		0.19				7	-89.41	1.63
	0.19	-0.34			0.06			7	-89.67	2.16
	0.29							5	-92.42	2.94
	0.22	-0.33	0.14	0.13				8	-88.87	3.02
	0.30		0.21					6	-91.29	3.03
	0.21	-0.36		0.23			0.13	8	-88.89	3.07
	0.19	-0.32	0.17			-0.05		8	-88.97	3.23
	0.20	-0.32	0.16		0.02			8	-89.02	3.32
Male	-0.27				0.43			6	-122.62	0.00
	-0.27		0.14		0.37			7	-122.19	1.47
	-0.24	-0.10			0.42			7	-122.42	1.93
	-0.30	-0.13			0.52	-0.27		8	-121.23	1.94
	-0.24			0.09	0.42			7	-122.59	2.26
	-0.24	-0.07	0.13		0.37			8	-122.09	3.66
	-0.24		0.14	0.07	0.37			8	-122.16	3.81
	-0.22	-0.13		0.25	0.51	-0.30		9	-120.98	3.89
	-0.30	-0.11	0.10		0.48	-0.25		9	-121.05	4.04
	-0.21	-0.10		0.08	0.41			8	-122.39	4.25

Table S3.6. The top ten best-supported models for the effect of parasite load on the proportion of time spent in diving behaviour per day for male and female European shags

	(Int)	Parasite load	Brood Size	Mean pop. Prod	Brood Age	Parasite load * Mean prod.	Parasite load * Brood Size	df	logLik	ΔAIC
Female	0.35				0.23			6	-93.06	0.00
	0.33			-0.13	0.24			7	-92.91	2.08
	0.40	0.07			0.21		-0.25	8	-91.69	2.10
	0.35		0.02		0.22			7	-93.04	2.34
	0.35	0.01			0.23			7	-93.06	2.39
	0.36	0.08		-0.15	0.23	0.19	-0.29	10	-89.31	2.44
	0.31	0.00		-0.10	0.25	0.18		9	-91.19	3.62
	0.37	0.08		-0.18	0.22		-0.26	9	-91.38	4.00
	0.30							5	-96.24	4.04
	0.32		0.03	-0.15	0.23			8	-92.86	4.44
Male	-0.27				0.41			6	-107.05	0.00
	-0.33			-0.20	0.46			7	-105.99	0.21
	-0.26		0.13		0.34			7	-106.36	0.95
	-0.33		0.14	-0.24	0.40			8	-105.21	1.05
	-0.21	-0.12			0.39			7	-106.49	1.20
	-0.29	-0.11		-0.23	0.43			8	-105.61	1.85
	-0.20	-0.12	0.13		0.33			8	-105.70	2.03
	-0.29	-0.11	0.14	-0.26	0.37			9	-104.74	2.55
	-0.23	-0.12			0.42		-0.05	8	-106.43	3.48
	-0.19	-0.12	0.09		0.35	0.11		9	-105.23	3.54

Table S3.7. The top ten best-supported models for the effect of parasite load on the proportion of time spent in resting behaviour per day for male and female European shags

	(Int)	Parasite load	Brood Size	Mean pop. Prod	Brood Age	Parasite load * Mean pop. Prod	Parasite load * Brood Age	df	logLik	ΔAIC
Females	-0.44				-0.26			6	-92.31	0.00
	-0.48	-0.09		0.10	-0.27	-0.23	0.33	10	-87.43	0.18
	-0.45			0.12	-0.28			7	-91.81	1.39
	-0.47	-0.03			-0.23		0.28	8	-90.73	1.68
	-0.44	-0.01		0.06	-0.31	-0.23		9	-89.54	1.82
	-0.44		-0.07		-0.24			7	-92.15	2.06
	-0.43	0.04			-0.26			7	-92.27	2.31
	-0.50	-0.10		0.16	-0.26		0.32	9	-89.88	2.50
	-0.48	-0.09	-0.03	0.11	-0.27	-0.22	0.32	11	-87.40	2.79
	-0.44		-0.09	0.16	-0.26			8	-91.54	3.29
Males	0.37				-0.45			6	-110.50	0.00
	0.38		-0.19	0.20	-0.43			8	-108.24	0.22
	0.38			0.15	-0.48			7	-109.59	0.53
	0.37		-0.13		-0.41			7	-109.85	1.04
	0.34	0.11			-0.45			7	-110.01	1.36
	0.35	0.08	-0.18	0.19	-0.43			9	-107.93	2.04
	0.35	0.09		0.14	-0.48			8	-109.23	2.19
	0.34	0.10	-0.13		-0.41			8	-109.36	2.46
	0.33	0.12			-0.50		0.09	8	-109.76	3.26
	0.34	0.10	-0.08		-0.41	-0.15		9	-108.68	3.54

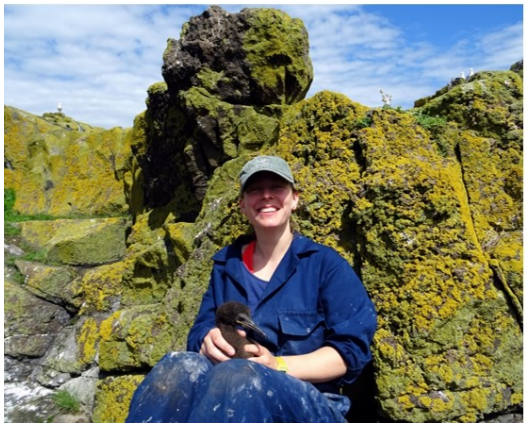
Table S3.8. The top ten best-supported models for the effect of parasite load on Daily energy expenditure for male and female European shags

	(Int)	Parasite load	Brood Size	Mean pop. Prod	Brood Age	Parasite load * Brood Age	df	logLik	ΔAIC
Female	0.34				0.29		6	-91.46	0.00
	0.34		-0.13		0.33		7	-90.73	0.93
	0.34			-0.02	0.29		7	-91.41	2.30
	0.34	0.02			0.29		7	-91.45	2.36
	0.37	0.06	-0.16		0.30	-0.27	9	-89.25	2.93
	0.36	0.06			0.26	-0.22	8	-90.51	2.94
	0.34		-0.15	0.04	0.33		8	-90.66	3.24
	0.34	0.00	-0.13		0.33		8	-90.73	3.37
	0.34	0.02		-0.03	0.29		8	-91.40	4.72
	0.36	0.07		-0.05	0.26	-0.23	9	-90.34	5.10
Male	-0.27				0.40		6	-122.82	0.00
	-0.26		0.13		0.35		7	-122.21	1.11
	-0.27			-0.09	0.42		7	-122.51	1.69
	-0.26		0.18	-0.14	0.37		8	-121.49	2.05
	-0.28	0.04			0.39		7	-122.78	2.24
	-0.26	0.01			0.48	-0.16	8	-122.09	3.25
	-0.27	0.05	0.14		0.35		8	-122.13	3.33
	-0.28	0.05		-0.10	0.41		8	-122.42	3.91
	-0.28	0.06	0.18	-0.15	0.36		9	-121.33	4.17
	-0.26	0.02	0.12		0.43	-0.14	9	-121.58	4.66

Chapter 4

The role of parasitism in the energy management of a free-ranging bird






The role of parasitism in the energy management of a free-ranging bird

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Authors' contributions

OH, SB, FD and JG designed the study. OH, SB, FD and MN collected the data. OC and CP performed the hormone assays and helped with the interpretation of the data. OH processed the accelerometry data, conducted the statistical analyses and wrote the manuscript. All authors (OH, SB, FD, MN, OC, CP and JG) contributed to interpreting results and improvement of this paper.

Abstract

Parasites can drive a large array of fitness-related traits and often prompt sub-lethal costs to the host by eliciting immune or stress responses. These sub-lethal costs can be hard to quantify but are crucial to our understanding of their host's ecology. Energy is a fundamental currency to quantify these sub-lethal fitness costs of parasitism, as energy is a limited resource and energetic trade-offs often exist between key fitness-related processes.

Daily energy expenditure (DEE) comprises of resting metabolic rate (RMR) and metabolic scope, the energy actualised for activity. These components are linked via the energy management strategy of an organism. Parasitism may play a role in the balance between self-maintenance and activity, as immune costs of parasitism can be expressed in elevated RMR. Therefore, understanding the relationship between DEE and RMR in the presence of parasitism may help with elucidation of individual energy management strategies.

Using a gradient of natural parasite load and proxies for RMR and DEE in a wild population of breeding European shags (*Phalacrocorax aristotelis*), we tested the effect of parasitism on maintenance costs as well as the relationship between RMR and DEE under the high energetic demands of reproduction.

We found a positive relationship between parasite load and RMR in females but not males and no relationship between RMR and DEE. This provides evidence for increased maintenance costs in individuals with higher parasite loads and indicates that European shags operate under an energy ceiling using an allocation energy management strategy, whereby a heightened immune response to parasitism creates restrictions on the allocation of energy to other activities.

This trade-off between self-maintenance and activity costs to safeguard DEE is likely to have fitness consequences in females as costs allocated to immune response trade-off against reproductive investment. Our findings demonstrate that understanding energy management strategies alongside fitness drivers is central to understanding the mechanisms by which these drivers influence energy use and allocation to optimise individual fitness.

Introduction

Parasites incur major fitness consequences to a huge array of taxa (Booth et al., 1993; Gooderham and Schulte-Hostedde, 2011; Reed et al., 2008b). Often parasites prompt sub-lethal effects to the host by eliciting immune or stress responses (Lettini and Sukhdeo, 2010; Martin et al., 2003; Sheldon and Verhulst, 1996; Smyth and Drea, 2016). However, the costs of these sub-lethal effects can be hard to quantify and consequently their impact on individuals and populations is often neglected in ecological research (Binning et al., 2017). Additionally, the mechanisms underlying these fitness responses and individual variation in responses are not well understood. Ignoring these effects of parasites reduces our understanding of their host's ecology, as we know parasites can drive a large array of fitness-related traits (Boulinier et al., 2016; Hamilton and Zuk, 1982; Norris and Evans, 2000a; Reed et al., 2008b; Sheldon and Verhulst, 1996).

Energy is a limited resource, and individuals are required to allocate energy to the demands of competing life-history traits to maximize fitness (Stearns, 1992). Therefore, trade-offs may exist in energetic terms between key fitness-related behaviours and processes and so energy is a central mechanism by which fitness responses to parasitism are manifested. A fundamental potential trade-off exists between basal or resting metabolic rate (RMR) and metabolic scope because ceilings exist on the sum of these two components, the daily rate of energy expenditure (DEE) (Elliott et al., 2014b; Mathot and Dingemanse, 2015; Welcker et al., 2010). For endotherms, RMR represents the minimum energetic cost of living during thermo-neutral rest (Mathot and Dingemanse, 2015; Welcker et al., 2015) and therefore largely represents the cost of self-maintenance, including immune activity (Burton et al., 2011). Metabolic scope is the remaining energy available to allocate to activity (Mathot and Dingemanse, 2015). DEE, RMR and metabolic scope are linked different ways depending on which energy management strategy the animal is operating under namely the performance, independent or allocation strategies (see Figure. 4.1. for details). A recent review of energy management in birds and mammals suggests that no single energy management strategy operates across all

species (Portugal et al., 2016).

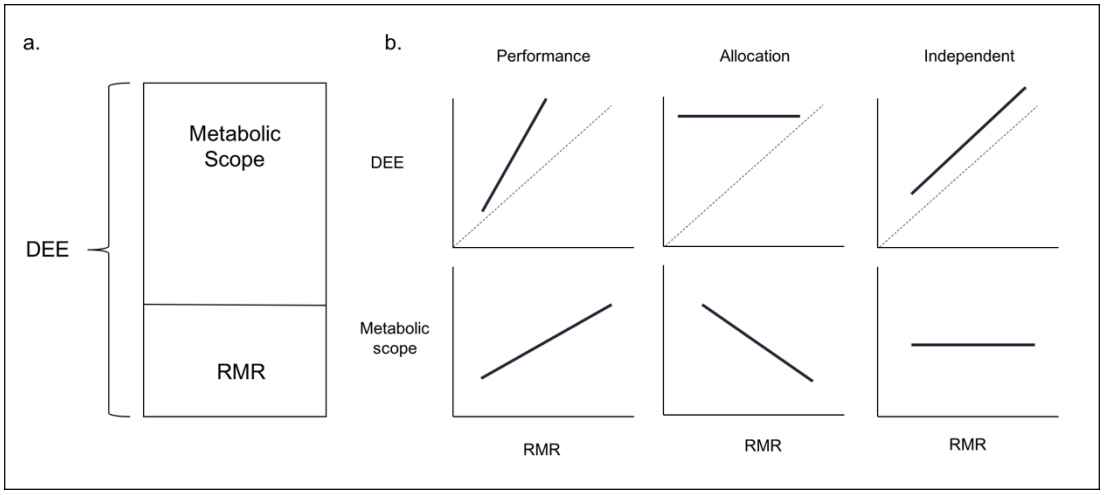

Figure 4.1. Graphical representation of energy budgets and possible energy management strategies. a. Daily energy expenditure (DEE) is the sum of Resting metabolic rate (RMR) and metabolic scope. b. Three theorised energy management strategies in graphical form adapted from Mathot & Dingemanse, (2015) and Careau & Garland, (2012) showing the relationship between Resting metabolic rate (RMR) and Daily energy expenditure (DEE) and metabolic scope under the three scenarios, the 1-to-1 line indicates a situation under which metabolic scope is zero for ease of interpretation of the slope of the lines.

Parasitism may play a central role in the relationship between self-maintenance and activity, as a primary predicted cost of parasitism is to RMR, through immune associated costs (Svensson et al., 1998). Hosts should invest in immunity to reduce the impacts of parasitism, particularly in long-lived species, where in contrast to short-lived species, survival contributes more to fitness and individuals should be selected to allocate more resources to a rapid, strong immune response to combat cumulative damage from parasites and to protect future reproductive success (Lochmiller and Deerenberg, 2000; Moe et al., 2007). Under the allocation model of energy management, this investment would create a trade-off between immune activity and other essential fitness-related organismal functions (Råberg et al., 2000; Sheldon and Verhulst, 1996). Under the performance model, there would be no trade-off with increased RMR, but instead metabolic scope and thus DEE would increase. Likewise under the independent model, despite no relationship between RMR and metabolic scope which would be unaffected, an increase in RMR results in an equivalent increase in DEE, since this is the sum of metabolic scope and RMR (Careau and Garland

Jr, 2012; Mathot and Dingemanse, 2015). The lack of a trade-off would itself likely have implications for fitness, and animals might ultimately reach maximal or optimal limits of DEE (Careau and Garland Jr, 2012; Mathot and Dingemanse, 2015).

Understanding the impacts of natural parasite burdens on individual metabolic rate is crucial, though this can be challenging due to difficulty in measuring natural parasite loads, particularly endo-parasites in wild populations. Experimental studies suggest endo-parasites and elevated immune function incur significant costs to hosts (Albon et al., 2002; Newborn and Foster, 2002; Sheldon and Verhulst, 1996). However there has been little work on the role of parasitism in mediating the energy management of individuals despite this being fundamental to the understanding of the fitness effects of parasitism.

Here we measure proxies of both DEE and RMR in individuals with known natural endo-parasite loads in a free-living population of breeding European shags (*Phalacrocorax aristotelis*) to understand the effect of endo-parasites on maintenance costs as well as the relationship between maintenance and total energy expenditure during the breeding period under high energetic demands. Specifically, we investigate two questions: 1. Does parasite load relate to RMR? We expect that individuals with higher parasite loads will have higher immune costs and that this will be reflected in elevated RMR 2. Does DEE relate to RMR and what can this tell us about the allocation between energy to reproduction and self-maintenance of this species? We predict that individuals will be energetically constrained by the provisioning of rapidly growing chicks during the chick rearing period under the allocation model and therefore any increase in RMR will not result in an increase in DEE.

Materials and Methods

Study site and species

The study was carried out at the Isle of May National Nature Reserve, south-east Scotland (56°11'N, 2°33'W) during the breeding seasons of 2015-2017. All individuals were part of a long-term population study and are individually

marked with a metal and a darvic ring for identification and accurately aging individuals that were first ringed as chicks.

Previous sampling of this population has shown a high prevalence of the nematode parasite *Contracaecum rudolphii* (Burthe et al., 2013; Granroth-Wilding et al., 2014; Reed et al., 2008b) in European shags, though parasite loads vary markedly between individuals (Burthe et al., 2013; Granroth-Wilding et al., 2014; Reed et al., 2012). Shags become infected with third stage larvae via their fish diet. Larval worms moult to become sexually mature adults which attach to the lining of the proventriculus and lower oesophagus in the final seabird host (Abollo et al., 2001; Burthe et al., 2013).

Measuring resting metabolic rate

Due to the difficulty of measuring metabolic rate in the field (Wilson et al., 2006b), Thyroid hormone plasma concentrations are increasingly being used as a proxy for resting metabolic rate in free ranging animals. The role of thyroid hormones in energy metabolism in birds in particular is now well established (Chastel et al., 2003b; Elliott et al., 2013b; Welcker et al., 2013). Triiodothyronine hormone (T3) especially is considered one of the major controllers for the regulation of tissue oxygen consumption and metabolic activity in endotherms (McNab, 1997). Studies carried out both in the laboratory and the field show close relationships for a range of species and life-history strategies.eg (Chastel et al., 2003b; Elliott et al., 2013b; Vézina et al., 2009; Welcker et al., 2013; Zheng et al., 2014). Therefore using T3 hormone as a proxy for RMR allows for approximation of individual measurements without the confounding effect of stress of capture associated with respirometry (Welcker et al., 2015).

Adult European shags were captured on the nest during chick rearing (when the chicks were between 5 and 36 days old) using a crook on the end of a long pole. At capture a 1 mL blood sample was collected from the brachial vein using a heparinized syringe and a 25-gauge needle. To avoid an effect of handling stress on hormone concentrations, sampling took place within 3 min of capture. Blood samples were taken between 03:30 and 07:30, before shags left for their first foraging trip of the day, and therefore birds were assumed to be post-

absorptive, which was later confirmed by endoscopy. Blood samples were stored on ice in the field. Whole blood was centrifuged and plasma and red blood cells were kept frozen at −20 °C until subsequent analyses.

Total 3, 3'-Triiodo-L-Thyronine (T3) hormone assays were performed at the Centre d'Etudes Biologiques de Chizé (CEBC), France. T3 analyses were performed using a single radioimmunoassay (RIA) and Total Thyroid hormone levels were assessed in duplicate without extraction as in Chastel et al., (2003) and Welcker et al., (2013). 25 µL of plasma was incubated with 10000 cpm of 125I-hormone (Perkin Elmer, US) and polyclonal rabbit antiserum (Sigma Aldrich, US). The bound fraction was separated from the free fraction by addition of a sheep anti-rabbit antibody. After overnight incubation and centrifugation, the bound fraction activity was then counted on a Wizard 2 gamma counter (Perkin Elmer, US). For quality control, reference materials were used and were within the acceptable range determined by the laboratory. Furthermore, every plasma was run in duplicates, samples with a coefficient of variance (CV) higher than 12% were re-assayed. Inter- and intra-assay variations were respectively 14.34% and 7.75%. Cross-reactions of T3 antiserum were as follows: triiodoD-thyroacetic acid 6%, L-thyroxine 0.2%, diiodo-L-thyrosine <0.01%, monoiodo-L-thyrosine <0.01%. Cross-reactions of T4 antiserum were as follows: triiodothyronine 4%, diiodo-L-thyrosine <0.01%, monoiodo-L-thyrosine <0.01%.

Quantifying parasite load

Worms were counted visually using the endoscope video screen (for detailed endoscopy methods see Burthe et al. (2013)). Burdens higher than 40 worms were hard to quantify in the field accurately and these were counted retrospectively via endoscopy video footage. Quantification of parasite burdens has been found to be repeatable within an individual across a season (Burthe et al. 2013). All endoscopy and blood sampling was performed by trained personnel (S. Burthe) holding a personal licence, and under a project licence issued by the UK Home Office.

Estimating daily energy expenditure

Tri-axial accelerometers (little Leonardo D3GT, AXY3 and Gulf Coast Data Concepts X8) were used to measure daily energy expenditure of a subset of the birds used for the hormone assay. Accelerometers were set to record at 25 or 50 Hz and attached on the midline of the mid back of individuals (as close to the centre of gravity as possible) using Tesa tape. All birds were successfully recaptured and accelerometers were retrieved after three days of deployment. Energy expenditure was calculated for diving, flying and resting (the main activities of European shags (Sakamoto et al., 2009)) using behaviour specific calibrations (see Hicks et al., 2017 for behaviour specific calibrations and detailed methods). These data were then used to calculate total Daily energy expenditure (DEE), the sum of the energetic costs of all behavioural bouts within 24-hour periods of activity (see Hicks et al 2018 for full details). Adult RMR and DEE have been found to vary with mass and reproductive stage and age in many seabirds (Elliott et al., 2014b; Green et al., 2013; Grémillet, 1997; Weimerskirch et al., 1995). Thus, adult mass and age were recorded at endoscopy and chick age at time of sampling was later back-calculated from wing length at ringing (Granroth-Wilding et al., 2014).

Statistical Analysis

All models were fitted separately for males and females due to non-independence of nest pairs. To answer the first question of whether parasite load is related to elevated RMR, we modelled T3 concentration using linear mixed effects models (LMMs). Parasite load, brood size (number of chicks), brood age (age of the oldest chick in the brood at the time of sampling), year and adult age and body mass were explanatory variables and we controlled for variation between birds and repeated sampling by including individual as a random factor. We fitted models containing all combinations of the fixed effects, and interactions between parasite load and brood age and parasite load and brood size (see Table 1 for explanations of model terms).

Secondly to understand how variation in RMR is related to DEE, we modelled DEE as a function of T3 concentration (a proxy for RMR) using linear mixed

effects models (LMMs). Adult age, adult body mass, brood age (at the time of sampling) brood size and year were also included as explanatory variables (Table 4.1). We controlled for variation between birds and repeated sampling by including individual as a random factor. All models were fitted using the lme4 package in R (Bates et al., 2014; R Core Team, 2015) and model selection for both sets of models was based on Akaike's information criterion (AIC) (Burnham and Anderson, 2001).

Table 4.1. Response and explanatory variables in models used. Explanation of each variable as well as units included

	Variable	Units	Code	Explanation
Response	Thyroid hormone concentration	ng/mL	T3	Concentration of total Triiodothyronine hormone in blood samples from adult shags. Hormonal proxy for RMR
	Daily Energy Expenditure	L/day	DEE	Total energy expenditure per 24 hour estimate by accelerometry and converted to rate of oxygen consumption via published calibrations for this species
Explanatory	Parasite load	worms	Para	Number of nematode worms in individual quantified with endoscope
	Brood Size	chicks	B.size	Number of chicks in nest may affect the energetic constraint adults are under in terms of provisioning
	Brood Age	days	B.age	Age of the oldest chick in the brood in days when the logger was deployed, may affect the energetic constraint adults are under as larger chicks need more food.
	Body mass	g	mass	Body mass of the adult sampled; body mass is found to relate to Thyroid hormone levels in some studies
	Age	years	Age	Age of the adult in years, this may have effects on thyroid hormone levels The study incorporated a full range of ages (Males 3-20, Females 4-14)
	Year		Year	Year in which the data was collected may have effects on thyroid hormone levels. Year is included as a factor to account for this variation

Results

Relationship between parasite load and RMR

A total of 87 (48 males and 39 females, 2015:8, 2016:42, 2017:37) individuals were blood sampled and endoscoped over three years (of which 27 were sampled in more than one year). The sample size in 2015 was much smaller than the other years, however, removal of these data had no impact on the

conclusions of the analyses therefore they were included. The best supported model for females showed a positive relationship between parasite load and T3 concentration, an effect of year, and an interaction between brood age and parasite load in that there was a more positive relationship between parasite load and T3 when broods were younger (See Figure 4.2 and Table 4.2). When year and brood age are accounted for, T3 concentration increased by 150% across the range in natural parasite load. The best supported model for males showed no effect of parasite load on T3 concentration. There was a negative effect of age as well as an effect of year on T3 concentration in males (see Figure 4.3 and Table 4.2).

Table 4.2. The top ten best supported modes selected by AIC of a linear mixed effect model to explain the effect of Parasite load and other variables on RMR (T3 concentration) for females and males.

	(Int)	Age	mass	Para	B. Size	B. Age	Year	Para * B.size	Para * B. Age	df	log link	Δ AIC
Female	-0.11			0.53		-0.29	+		-0.35	8	-40.23	0.00
	-0.11	-0.17		0.52		-0.29	+		-0.34	9	-39.66	0.87
	-0.26		-0.23	0.56		-0.37	+		-0.46	9	-39.74	1.02
	-0.27	-0.18	-0.24	0.54		-0.37	+		-0.46	10	-39.11	1.77
	-0.10			0.53	-0.05	-0.29	+		-0.37	9	-40.16	1.87
	0.24			0.64		-0.17	+			7	-42.44	2.43
	0.39			0.68			+			6	-43.45	2.45
	-0.11	-0.17		0.51	-0.05	-0.28	+		-0.36	10	-39.59	2.72
	-0.26		-0.24	0.55	-0.06	-0.37	+		-0.49	10	-39.66	2.86
	-0.09			0.59	-0.08	-0.33	+	-0.20	-0.37	10	-39.68	2.91
Male	-0.12	-0.40					+			6	-56.57	0.00
	-0.32	-0.37	0.33	-0.17			+			8	-54.66	0.17
	-0.31	-0.39	0.26				+			7	-55.75	0.37
	-0.10	-0.38		-0.13			+			7	-55.92	0.71
	-0.39	-0.39	0.38	-0.20		0.11	+			9	-54.43	1.72
	-0.11	-0.39				-0.04	+			7	-56.54	1.94
	-0.13	-0.40			-0.02		+			7	-56.56	1.98
	-0.33	-0.37	0.34	-0.16	-0.02		+			9	-54.65	2.15
	-0.35	-0.39	0.28		-0.06		+			8	-55.66	2.18
	-0.31	-0.39	0.27			0.01	+			8	-55.75	2.37

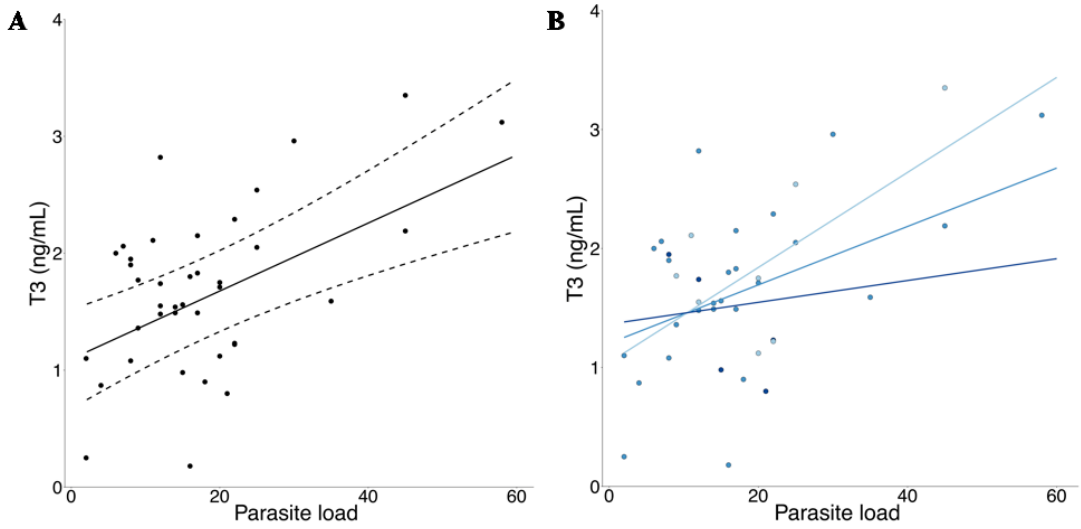

Figure 4.2. Relationship between parasite load and plasma total 3, 3'-Triiodo-L-Thyronine (T3) concentration (a proxy for Resting metabolic rate) in female European shags Data points represent individual T3 concentrations, the predicted lines from the best supported model (solid lines) and their 95% confidence intervals (dashed). A shows the relationship between parasite load and T3 concentration when year and brood age are accounted for, B shows the interaction between parasite load and brood age in relation to T3 concentration. Solid lines represent predicted lines from the best supported model under different brood age scenarios -1 Standard deviation of the mean brood age (light blue) Mean brood age (mid blue), and +1 Standard deviation of the mean (dark blue).

Relationship between RMR and DEE

Daily energy expenditure was measured in a subset of the birds (71 individuals; 2015=3, 2016=40, 2017=28) to understand the relationship between RMR and DEE. The best supported model for females was the null model with no relationship found between T3 and DEE (see Figure 4.4 and Table 4.3). For males, the best supported model showed a negative effect of body mass on DEE and no effect of T3 (see Figure 4.4 and Table 4.3). However, the null model is also equally well supported (within Δ 2 AIC of the top model).

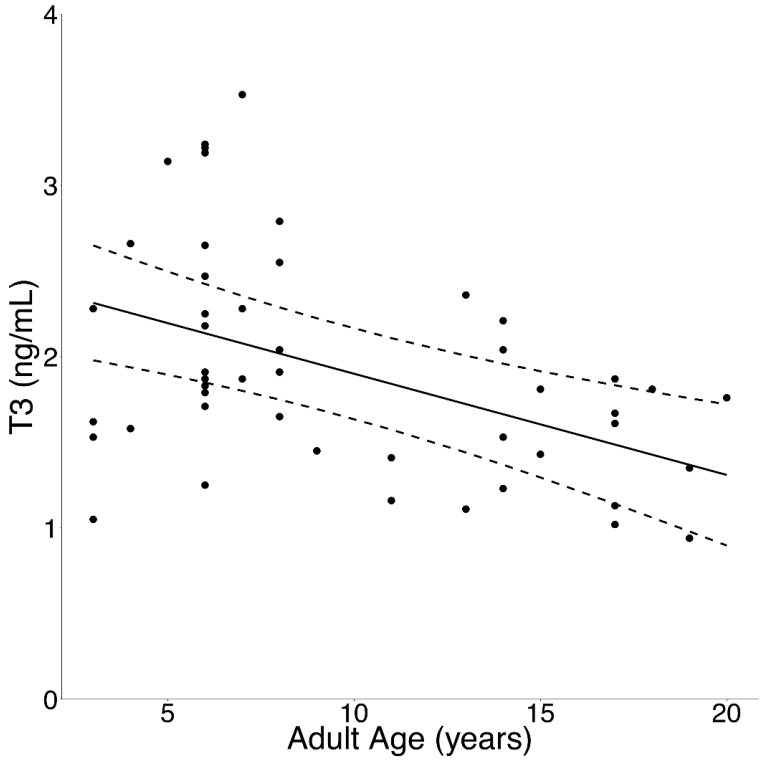

Figure 4.3. Relationship between adult age and plasma total 3, 3'-Triiodo-L-Thyronine (T3) concentration (a proxy for Resting metabolic rate) in male European shags. Data points represent individual T3 concentrations, the predicted lines from the best supported model (solid lines) and their 95% confidence intervals (dashed).

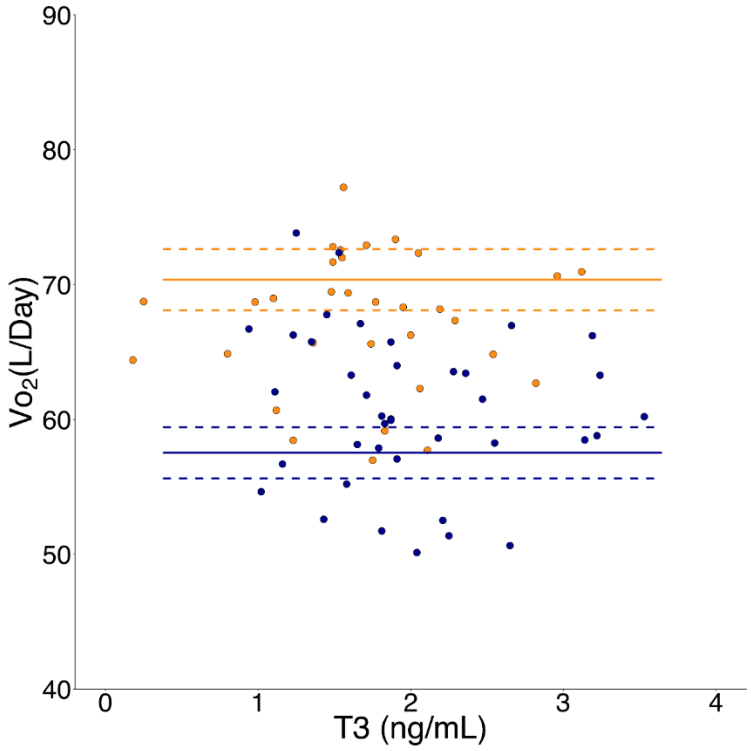

Figure 4.4. Relationship between DEE and plasma total 3, 3'-Triiodo-L-Thyronine (T3) concentration (a proxy for resting metabolic rate) in European shags. Dots represent individual T3 concentrations, the predicted lines from the best supported model (solid lines) and their 95% confidence intervals (dashed) are presented for males (blue) and females (Orange)

Table 4.3. The top ten best supported modes selected by AIC from a mixed effect linear model to explain the relationship between Daily Energy Expenditure calculated by accelerometry and RMR (measured by a proxy; plasma concentration of T3 hormone) for female and male European shags.

	(Int)	Age	mass	T3	B. Size	B. Age	Year	df	log link	Δ AIC
Female	0.58							3	-38.18	0.00
	0.41						+	4	-37.29	0.22
	0.10		-0.52					4	-37.39	0.41
	-0.12		-0.55				+	5	-36.40	0.43
	0.57					0.20		4	-38.14	1.91
	0.42					0.17	+	5	-37.61	2.85
	0.19		-0.41			0.17		5	-37.68	2.99
	0.57	-0.07						4	-38.71	3.06
	0.39	-0.08					+	5	-37.79	3.21
	0.59				-0.14			4	-38.80	3.23
Male	-0.18		-0.50					4	-49.03	0.00
	-0.54							3	-50.27	0.49
	-0.15		-0.59		0.39		+	7	-47.20	2.34
	-0.15		-0.51		0.17			5	-49.37	2.68
	-0.53				0.15			4	-50.78	3.50
	-0.20		-0.47			0.09		5	-49.90	3.73
	-0.24		-0.48				+	6	-48.97	3.89
	-0.54					0.12		4	-50.98	3.90
	-0.17	-0.02	-0.50					5	-49.99	3.92
	-0.18		-0.48	-0.05				5	-50.05	4.03

Discussion

We found a positive relationship between natural parasite load and a hormonal proxy for Resting Metabolic Rate (RMR) across multiple years in female European shags, suggesting increased maintenance costs in individuals with higher parasite loads. Host maintenance costs are likely to be increased in response to parasitism due to increased cell repair and cost of dealing with the parasite infection as well as the immune response itself (Lochmiller and Deerenberg, 2000). This combined with a lack of relationship between our proxy for RMR and Daily Energy Expenditure (DEE) suggests that female European shags operate under an allocation energy management strategy. Therefore a heightened immune response to parasitism creates restrictions on the allocation of energy to other activities and is likely associated with subsequent fitness consequences.

We found no relationship between RMR and parasitism in males despite the strong relationship found in females. Males are hypothesised to experience the immunosuppressive qualities of testosterone (Grossman, 1985), thus the lack of relationship between RMR and parasitism may be due to suppression of their parasite-induced immune response. Additionally, trade-offs between reproduction and self-maintenance are especially strong in female shags, who have higher DEE than males on average (see Figure 4.3. and Hicks et al 2018). Females across many taxa often invest more in reproduction than males (Clutton-Brock, 1991) meaning that females may be closer to their optimum energy expenditure than males. Thus the greater energetic constraint of females to extrinsic and intrinsic conditions could explain these sex differences in response to parasitism (Hicks et al., 2018; Lewis et al., 2015).

Using T3 concentration as a proxy for RMR enables us to gain greater insight into the energy use of free-ranging animals and complements other approaches. Hicks et al. (2018) show that time spent in flight for European shags reduced with higher parasite loads, but that this decrease was more than expected based on the cost of this behaviour alone. The results from this study suggest that the reduction in flight duration observed in heavily parasitised birds may be due to compensation for increased maintenance costs as well as the increased cost of flight. For European shags it is not possible to quantify the extent of this compensation as we have yet to calibrate T3 concentration against RMR using respirometry. However T3 plasma concentration is increasingly being used as a proxy for RMR due to strong correlations found in multiple species and the reduction in the confounding effect of stress of capture associated with respirometry (Blackmer et al., 2005; Bouwhuis et al., 2011; Chastel et al., 2003b; Elliott et al., 2013b; Moe et al., 2007; Welcker et al., 2013; Welcker et al., 2015). In an experimental study, a 50% increase in free T3 corresponded to a 50% increase in RMR in kittiwakes (Welcker et al., 2015). In this study, we found a 150% change in T3 concentration across the natural range of parasite load, meaning that the corresponding change in RMR to parasitism could be even greater than in previous experimental work.

It is likely that energy management is not fixed across time but varies according to particular conditions as seen in Hicks et al., (2018) where DEE increased with brood age but not in relation to parasite load. An adult's cumulative energetic investment in its brood increases with brood age (Drent and Daan, 1980). Thus the trade-off between allocation to reproduction and self-maintenance may also shift with brood age. In a young brood, when cumulative investment is lower than an older brood energy allocation to immune response may be beneficial to ensure that the next reproductive event is reached (Ilmonen et al., 2000), but may reduce parental effort (Råberg et al., 2000) and negatively affect offspring success. However when cumulative investment in a brood is higher investment to current reproduction may be prioritised. Interestingly, we found the positive relationship between RMR and parasite load is greater when the adult's chicks are younger.

At the scale of a lifetime life-history theory predicts trade-offs between reproduction and self-maintenance change with age. As the probability of future reproduction declines with age, it is predicted that resources will be increasingly allocated to current reproduction rather than maintenance (Herborn et al., 2015; van Noordwijk and de Jong, 1986). There is good evidence for this in short-lived species such as great tits and zebra finch (Bouwhuis et al., 2011; Moe et al., 2009), however in longer lived birds evidence is equivocal e.g. (Blackmer et al., 2005; Elliott et al., 2015; Moe et al., 2007). In this study we found a negative relationship between age and RMR in males, suggesting older individuals invest less energy in maintenance as in thick billed murres (Elliott et al., 2015). However, we found no effect of age on RMR in females, sex-specific senescence occurs in a number of wild vertebrate populations, possibly due to different trade-offs existing with age in males and females (Clay et al., 2018; Froy et al., 2013; Froy et al., 2017; Murgatroyd et al., 2018b).

We found no evidence for a relationship between RMR and DEE indicating that DEE does not vary with maintenance costs. Despite parasite induced behavioural costs, European shag DEE does not correlate with parasite load (Hicks et al., 2018) suggesting they experience optimal energy budgets. These results provide evidence for the allocation management strategy in this species,

indicative of strong limits or optimisation of DEE (Drent and Daan, 1980; Mathot and Dingemanse, 2015). As such, an increase in RMR will cause a reduction in metabolic scope, which may have consequences for fitness-related behaviours such as chick provisioning or foraging. Our findings are in agreement with studies providing evidence for the allocation management strategy in three seabird species (Blackmer et al., 2005; Elliott et al., 2014b; Welcker et al., 2015) but are contrary to other studies (including those of other seabird species) providing evidence for both performance and independent strategies (Chastel et al., 2003b; Portugal et al., 2016).

It is unclear why species vary in their energy management strategy, though during energetically demanding periods, the capacity to increase expenditure can impose energy limits making an allocation strategy more likely (Elliott et al., 2014b). Under limited energy it can be difficult to buffer the additional costs of parasites. Therefore, considering the role of parasitism in mediating energy allocation between reproduction and self-maintenance is crucial in understanding the mechanism of its fitness effects. The allocation strategy creates energetic trade-offs under an overarching constraint, yet before now we had little knowledge about what drives these trade-offs. Changes in energy allocation can influence reproduction in the short term, but in the long term there may be negative consequences of reducing allocation to self-maintenance (Blackmer et al., 2005). Therefore, quantifying energy management strategies alongside potential fitness drivers is crucial to understand the mechanisms by which they act and influence energy allocation to optimise individual success.

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Supplementary materials

Energy management strategies

Performance model: Variation in RMR reflects variation in the size of organs that mobilize energy (e.g., digestive organs, muscle). Therefore, individuals with higher maintenance MR are able to maintain higher levels of energy output (i.e., have higher DEE). This strategy predicts higher RMR to be associated with both greater metabolic scope and therefore when summed, a higher DEE. (Careau et al., 2008).

Allocation model: Variation in RMR does not reflect variation in the size of organs that mobilize energy; differences in RMR are therefore not associated with differences in DEE. Individuals with higher maintenance MR have less energy available to allocate to energetically costly behaviours and so are predicted to have lower expression of such traits (Mathot and Dingemanse, 2015).

Independent Model: Regulation of RMR and DEE are independent but an increase in RMR causes an increase in total energy expenditure as metabolic scope is also independent of RMR. Therefore, higher RMR is associated with higher DEE because while metabolic scope is unaffected it sums with an increasing RMR to drive an increased DEE.

Chapter 5

Sub-lethal effects of natural parasitism impact on maternal but not paternal reproductive success in a wild population

Sub-lethal effects of natural parasitism impact on maternal but not paternal reproductive success in a wild population

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Author contributions

OH, SB, FD, EC and JG designed the study. OH, SB, FD, and MN collected the data. OH processed the data, conducted the statistical analyses and wrote the manuscript. AB provided statistical advice. All authors (OH, JG, FD, EC, MN, AB and SB) contributed to interpreting results and improvement of this paper.

Abstract

Parasites are ubiquitous and exert strong ecological and evolutionary forces on populations. The effects of most parasites are sublethal, with consequences for vital rates such as reproduction. However, quantifying these costs can be challenging, due to difficulties in measuring natural parasite infections in wild populations, particularly for endoparastites where indirect assessments are inaccurate. Here we use endoscopy to directly quantify natural parasites in individuals over a seven year period in relation to reproductive success, in a wild population of seabirds. Despite significantly higher burdens in males, we only found substantial negative impacts of parasitism on breeding success in females. Female reproductive success, as measured by fledgling success, declined by 30% across the range of natural parasite burdens. In contrast, there was no overall effect of parasitism on male success despite their higher burdens; male breeding success varied with age, but there was no interaction between age and parasitism. These effects of parasitism persisted regardless of environmental conditions and when accounting inter-annual population differences in breeding success. Our results therefore provide both evidence and quantitative estimates of the profound sub-lethal effects of parasitism acting through different demographic components of the population, highlighting the potential for parasites to shape how ecological and evolutionary processes may act with complex implications for demography and selection.

Introduction

Inter-individual variability in fitness underlies ecological and evolutionary dynamics and is a key driver of demographic processes (Vindenes and Langangen, 2015). Understanding the drivers of individual differences in life-history is therefore crucial in understanding population growth and persistence (Agnew et al., 2000). Parasites can be one such driver; they are ubiquitous and exert strong ecological and evolutionary forces on their hosts (Lochmiller and Deerenberg, 2000) and can influence inter-individual differences in vital rates via sub-lethal effects.

Parasites can affect the condition, behaviour and energy use of the host (Binning et al., 2012; Hicks et al., 2018; Sheldon and Verhulst, 1996) yet it is hard to quantify these sub-lethal parasitic costs (Binning et al., 2017). Individuals, particularly from long-lived species with slow reproductive rates, should be selected to allocate a strong immune response to combat damage from parasites in order to protect future reproductive success (Lochmiller and Deerenberg, 2000). As such, trade-offs between investment in reproduction and immune response could be important in determining optimal life-history traits (Lochmiller and Deerenberg, 2000; Norris and Evans, 2000b). Indeed for animals operating under energetic constraints there is now evidence that individuals with high parasite burdens exert additional foraging costs and may be unable to invest as much into reproduction as individuals with lower parasite burdens (Hicks et al., 2018). However, no study has yet linked the mechanisms by which parasites cause sublethal costs with the consequences of such costs. Therefore making these links between individual level effects of intrinsic and extrinsic variables, such as parasitism, is a key step in understanding population change (Lewis et al., 2009).

Extrinsic and intrinsic variables, such as parasitism, can have differential individual impacts due to interactions between them (Daunt et al., 2014; Lewis et al., 2009). In mammals and birds, males are often more susceptible to parasites and have higher burdens than females (Poulin, 1996; Schalk and Forbes, 1997; Zuk and Stoehr, 2002). Furthermore, differential selection by parasitism on males and females can lead to different levels of resistance and

tolerance between sexes, such that males may suffer higher virulence and be more responsive to equivalent parasite burdens than females due to a number of physiological, hormonal or behavioural differences (Duneau and Ebert, 2012; Grossman, 1985; Poulin, 1996; Schalk and Forbes, 1997; Thompson et al., 2017; Zuk and Stoehr, 2002). The effects of parasitism are also known to be condition dependant and may vary within individuals over time (Bize et al., 2010; Reed et al., 2008b). Individual parasite burdens or responses to burdens may relate synergistically to environmental conditions, with impacts of parasitism more apparent when animals are operating under greater pressure in more marginal conditions (Laaksonen et al., 2002; Tompkins et al., 2011). It is therefore important to longitudinally test and quantify the impact of parasitism on individual reproductive success and how this may interact with varying environmental conditions.

Previous work on a long lived seabird, the European shag *(Phalacrocorax aristotelis)* has shown that experimental removal of parasites positively impacts reproductive output of females, but effects depended on individual status and environmental conditions (Granroth-Wilding et al., 2014; Reed et al., 2008b). Together, these suggest an interaction between intrinsic and extrinsic factors in determining the fitness effects of parasitism. Here, in the same species, we use seven years of individual natural parasite load and breeding success data to quantify the influence of parasite load on offspring production at an individual level in adult males and females. This unique approach allows us to look at the longitudinal effects of un-manipulated parasite burdens on individuals across a natural gradient of parasite loads. This is crucial in complementing previous experimental approaches as almost all individuals have parasites and thus total exclusion of parasites may not be relevant to this population. Focusing on a gradient of natural parasite loads, rather than whether individuals are treated or untreated also enables exploration of the potentially subtle effects of parasitism to individuals. Furthermore, repeat measurements over time of the same individuals removes some of the effects of individual quality confounding the results (Daunt et al., 2014). We specifically test three hypotheses: H1) Natural parasite load negatively impacts individual breeding success and H2)

Parasitism interacts with environmental conditions to drive breeding success, in that the effects of parasitism are more pronounced with poor environmental conditions H3) Parasitism effects differ between sexes, in line with previous work on this study system which suggests females are affected more than males (Hicks et al., 2018; Reed et al., 2008b).

Methods

Measuring parasite load and breeding success

The study was carried out on the Isle of May National Nature Reserve, south-east Scotland (56˚11'N, 2˚33'W) during chick rearing in the breeding seasons of 2011-2017. A total of 101 adult shags were endoscoped to determine natural parasite load of gastrointestinal nematodes *Contracaecum rudoliphii.* While 52 of these individuals were endoscoped across at least two years giving a total sample size of 220 observations (106 male, 114 female) (see Table S5.1 for details of sampling structure). Consistent with previous work, endoscopy revealed all adults were parasitized but that burdens varied both between individuals and within individuals across years, separating any correlation between individual quality and burdens. Worm burdens were counted visually using video images from the endoscope (for details see Burthe et al. 2013, Hicks et al. 2018). These methods provide a more representative measure of burden than indirect methods such as fecal egg counts which significantly underestimate levels of parasitism as they only detect sexually mature adult worms that are producing eggs (Granroth-Wilding et al., 2016). All individual shags were uniquely marked with a metal ring as chicks (therefore of known age) and sexed by vocalisation (Snow, 1960). Breeding success (number of chicks fledged per pair) was recorded. All endoscopy was performed by trained personnel (S. Burthe) holding a personal licence operating under a project licence issued by the UK Home Office under the Animals (Scientific Procedures) Act 1986. We used mean population productivity as an annual proxy for environmental conditions (as in Reed et al. 2008b, Granroth-Wilding et al. 2014, Bogdanova et al. 2014). This was calculated as the average number of fledged young per incubated nest in a series of un-manipulated, long-term monitoring

plots completely independent of the birds included in the parasite study. Throughout, we assume that reproductive success is a measure of fitness as it is an important contribution to life time reproductive success.

Statistical analysis

All models were generalized linear mixed models (GLMMs) with binomial error and logit link function fitted using the *lme4* package in R (Bates et al., 2014; R Core Team, 2015). The response variable in all models was a two columned combined variable of the number of chicks fledged and the number of chicks failed, representing 'fledging probability per egg'. We controlled for variation between birds and repeated sampling by including individual as a random factor. To test H1, that natural parasite burdens negatively impact the breeding success of their hosts, we use a likelihood ratio test to compare a model containing explanatory variables known to affect breeding success: age, quadratic effect of age (Jaeger et al. 2014) and year, which accounts for known inter-annual variability, against the same model with the addition of individual parasite load. To test H2, that parasite load interacts with extrinsic variables to impact breeding success, we considered a null model that contains parasite load, age, quadratic effect of age and variables which represent the extrinsic environment: mean population productivity, mean lay date and lag mean population productivity. We then used likelihood ratio tests to calculate the p-values associated with adding interactions between each of these variables and parasite load to the null model.

For both H1 and H2 we fitted models for males and females separately, due to non-independence of nest pairs, to directly quantify the impact of parasitism on the breeding success of both sexes. Comparison of results for males and females allowed us to test H3. P-values were calculated using likelihood ratio tests, as implemented in R using the 'anova' function and are provided for all analyses.

Results

Parasite loads included in these analyses ranged from 2 to 40 worms though these distributions differed between the sexes, males: mean: 23, SD±11.0 females mean: 16, SD±9.2, t(205)= -4.9 p= <0.0. Despite males being more heavily parasitised however, we found that individual fledgling success is negatively related to parasite load in females but not males. For females, the addition of parasite load to the model significantly improved the fit of the model (χ^2 = 4.56; p = 0.03) and the full range of parasite burdens, led to a 30% reduction in breeding success, equivalent to a loss of 0.7 chicks per adult (see Figure 5.1.). For males, the addition of parasite load did not improve the model (χ^2 = 0.15; p = 0.70) and although there was evidence for a significant quadratic effect of age on the number of chicks fledged (χ^2 = 7.46; p= 0.01) (see Figure S5.2.) there was no interaction between age and parasite burden on breeding success (Table 5.2.).

There was no interaction between parasitism and the prevailing environment in driving breeding success for either females or males) as including interactions between parasitism and any of the environmental variables did not improve the model fit (see Table 5.1 and Table 5.2).

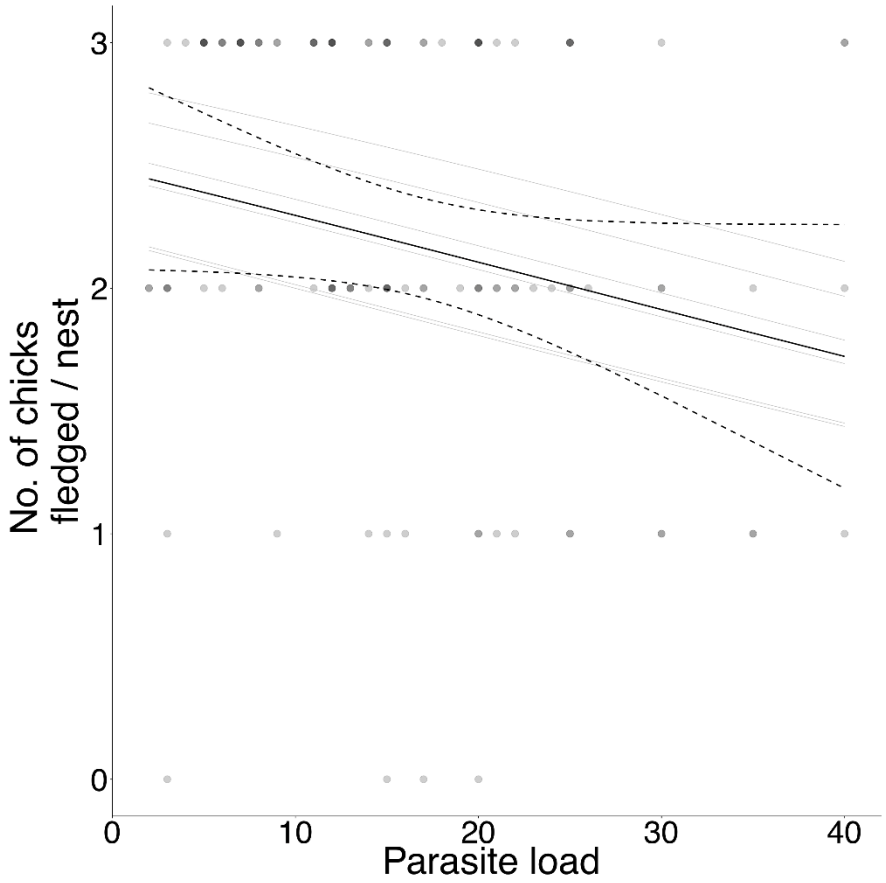

Figure 5.1. The effect of parasite load on breeding success (number of chicks raised per nest) in female European shags. Lines represent predicted lines from the best supported model (solid lines). Grey lines represent the predicted lines for each year of the study which vary in the mean population productivity (a proxy for environmental conditions), the black line represents the predicted line under the mean environmental conditions with 95% confidence intervals (dashed).

Table 5.1. The relationship between breeding success, parasite load and extrinsic variables in female European shags. Models predicting individual level breeding success (number of chicks fledged per nest), with response variables parasite load, mean population productivity, mean lag population productivity, mean lay date and adult age and the interactions between these variables and parasite load. Estimates from generalized linear mixed models of slopes and estimates are presented for all effects. P values were calculated using likelihood ratio tests. Significant terms are indicated in bold.

Explanatory variable	P value	Effect size	±SE	Hypothesis
Parasite load	**0.04**	-0.22	0.11	H1
Parasite load	**0.03**	-0.23	0.10	H2
Parasite load * Mean productivity	0.30	0.12	0.12	
Parasite load * Lag Prod	0.93	0.03	0.10	
Parasite load * Lay date	0.28	-0.16	0.12	
Parasite load * Age	0.45	-0.08	0.11	
Parasite load * Age^2	0.67	0.05	0.09	

Table 5.2. The relationship between breeding success, parasite load and extrinsic variables in male European shags. Models predicting individual level breeding success (number of chicks fledged per nest), with response variables parasite load, mean population productivity, mean lag population productivity, mean lay date and adult age and the interactions between these variables and parasite load. Estimates from general linear mixed models of slopes and estimates are presented for all effects. P values presented from ANOVAs between models with and without the term of interest. Significant terms are indicated in bold

Explanatory variable	P value	Effect size	±SE	Hypothesis
Parasite load	0.70	-0.04	0.11	H1
Parasite load	0.44	-0.08	0.11	H2
Parasite load * Mean productivity	0.22	-0.13	0.11	
Parasite load * Lag Prod	0.86	0.02	0.10	
Parasite load * Lay date	0.79	0.03	0.10	
Parasite load * Age	0.56	-0.07	0.11	
Parasite load * Age^2	0.82	-0.04	0.16	

Discussion

Despite males consistently having higher burdens than females, we found substantial negative effects of parasitism on reproductive success in female free-living shags but not in males. We found no evidence of the environment mediating the effects of parasitism. This means that the negative fitness effects of parasitism are additive and exist even under good conditions.

It is unusual to be able to show such clear fitness impacts of natural parasite burdens, thus most studies have taken an experimental approach (Albon et al., 2002; Hudson et al., 1998; Reed et al., 2008b; Watson, 2013). However we find a 30% decline in host fledgling success across the natural range of parasite load, which is important as it shows that natural ranges of parasitism existing in wild populations are exerting significant sub-lethal fitness effects. These costs act only on females, contrary to prediction that males are more susceptible to infection and develop higher parasite burdens and costs than females (Duneau and Ebert, 2012; Grossman, 1985; Poulin, 1996; Thompson et al., 2017; Zuk and Stoehr, 2002). However, this is consistent with previous work in this species, which provides experimental evidence for the effect of parasitism on reproductive output as well the energetic and behavioural costs of natural parasitism in females but not males (Hicks et al., 2018; Reed et al.,

2008b). Female shags also experience a larger effect of wind on their year round foraging behaviour than males (Lewis et al., 2015). In general, females consistently invest more than males in reproduction (Clutton-Brock, 1991; Kokko and Jennions, 2003; Trivers, 1972) and may consequently experience stronger energetic trade-offs than males (Hicks et al., 2018; Korpimaki et al., 1993; Reed et al., 2008b). Crucially these differing effects of parasitism on males and females may have may have significant implications for selection and population processes.

Immune costs are often context and host-condition dependent, with effects expected to be more apparent in bad conditions (Bize et al., 2010; Bustnes et al., 2006). Yet we found no evidence for an interaction between parasitism and environmental conditions in their effects on reproduction. This is crucial as it means the fitness effects of parasitism will not just impact individuals under poor conditions as predicted, but substantially reduce reproductive output across all years, and hence are having a greater impact on population dynamics than previously thought. However, it is important to note that over the study, conditions were always relatively good when compared to long term data from this population and the sample of individuals were more successful than the mean of the population (see supplementary materials Figure S5.1) thus across the whole population, the impacts of parasitism could be much greater than observed.

Parasites infect nearly every known animal taxon (Dobson et al., 2008), thus it is important to quantify their impacts on host fitness and population success. Critically we found no interaction between parasitism and environment in driving breeding success, meaning that parasites impact female reproduction even when conditions are good. Thus parasitism has a more substantial impact on population processes than previously thought (Watson, 2013). The next steps must be to incorporate these effects into population models, which depend on vital rates such as breeding success and survival but do not yet incorporate the effects of parasitism (Frederiksen et al., 2008).

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Supplementary materials

Table S5.1. The number of unique individual adult European shags sampled in multiple years over the duration of the seven year study. Sampling included measuring endo-parasite load using an endoscope technique and recording reproductive success (fledglings raised) for each individual.

No. years sampled	6	5	4	3	2	1
Count	4	4	15	9	20	49
Cumulative count	4	8	23	32	52	101

Table S5.2. The relationship between breeding success, parasite load and extrinsic variables in male and female European shags. Models predicting individual level breeding success (number of chicks fledged per nest), with response variables parasite load, mean population productivity, mean lag population productivity, mean lay date and adult age and the quadratic effect of age. Estimates from general linear mixed models of slopes and estimates are presented for all effects. P values presented from ANOVAs between models with and without the term of interest. Significant terms are indicated in bold

	Explanatory variable	P Value	Effect size	±SE
Females	Age	0.99	0.00	0.13
	Age^2	0.08	0.12	0.07
	Mean productivity	**<0.01**	0.52	0.17
	Lag productivity	0.31	-0.13	0.13
	Lay date	0.15	0.29	0.20
	Parasite load	**0.03**	-0.23	0.10
Males	Age	0.09	0.18	0.11
	Age^2	0.67	0.04	0.08
	Mean productivity	0.12	0.25	0.16
	Lag productivity	0.48	-0.09	0.13
	Lay date	0.49	0.13	0.18
	Parasite load	0.44	-0.08	0.11

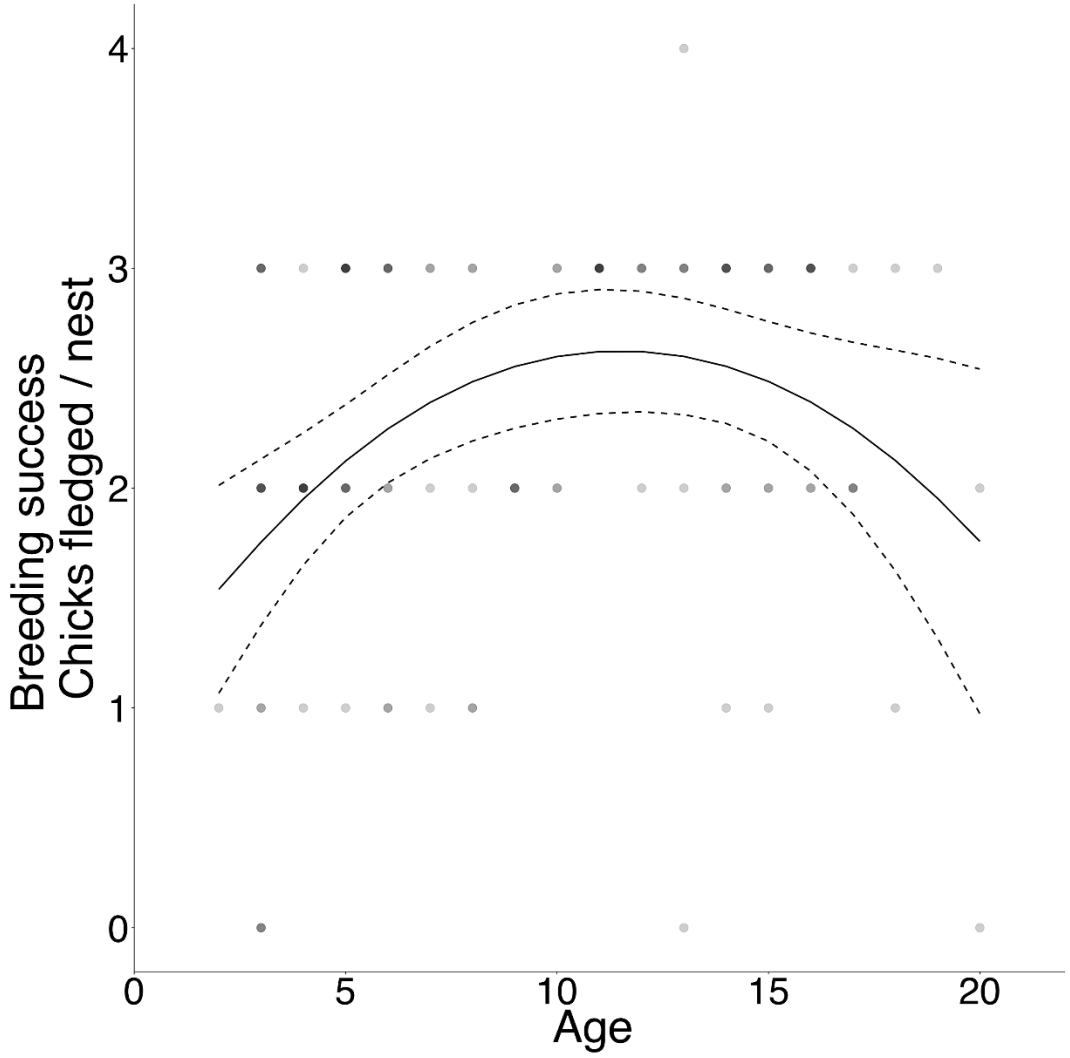

Figure S5.1. The effect of adult age on breeding success (number of chicks raised per nest) of male European shags. Lines show predicted lines (solid line) and their 95% confidence intervals (dashed) from the best supported model to describe individual variation in breeding success.

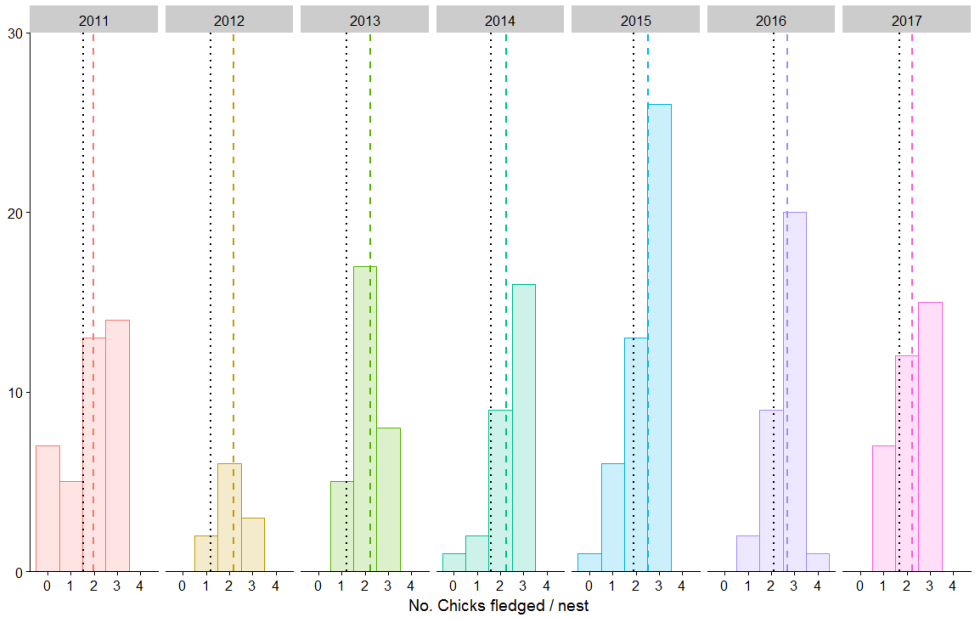

Figure S5.2. Comparison of individual breeding success with mean population breeding success. Density plot of breeding success of individuals in this study (coloured by year) with mean population productivity calculated from population wide study plots by year (black dotted line) and mean productivity of the individuals in this study (dashed lines coloured by year). Comparison of the mean breeding success of the individuals included in this study and the population study plots shows that the mean number of chicks raised by the adults in this study is always slightly higher than the population average.

Chapter 6

General Discussion

Key Findings

The main aim of this thesis was to quantify the effects of parasitism in a wild population of European shags to further our understanding of the mechanisms by which parasites affects individuals and populations. Using a number of techniques, we were able to understand the maintenance, energetic and behavioural costs of endo-parasites and the consequences of these natural parasite burdens on individual reproductive success.

Firstly, we generated accurate behavioural-specific calibrations of ODBA-derived oxygen consumption. This improved the usage of accelerometry to determine energetic costs of different behaviours in wild shags. Secondly, using these established calibration relationships, we found behavioural and energetic costs of parasitism to flight in female shags. Flight costs were higher in females with higher parasite loads and as a likely consequence, the duration of time spent in flight reduced with parasite load. Thirdly, using T3 hormone as a proxy for resting metabolic rate, we found that high parasite loads were associated with elevated resting metabolic rates in females but not males. We also found evidence for a fixed energy budget and the allocation energy management strategy, reinforcing that shags cannot simply increase their efforts in the face of parasitism. Finally, we found that parasite load is negatively correlated with breeding success in females but not males, presumably due to the combined energetic, behavioural and maintenance costs of parasitism to females, which inhibits their ability to provision young. Here I aim to bring the work of this thesis together and highlight implications, limitations and new emerging questions from the study, under various key themes summarised below in Figure 6.1.

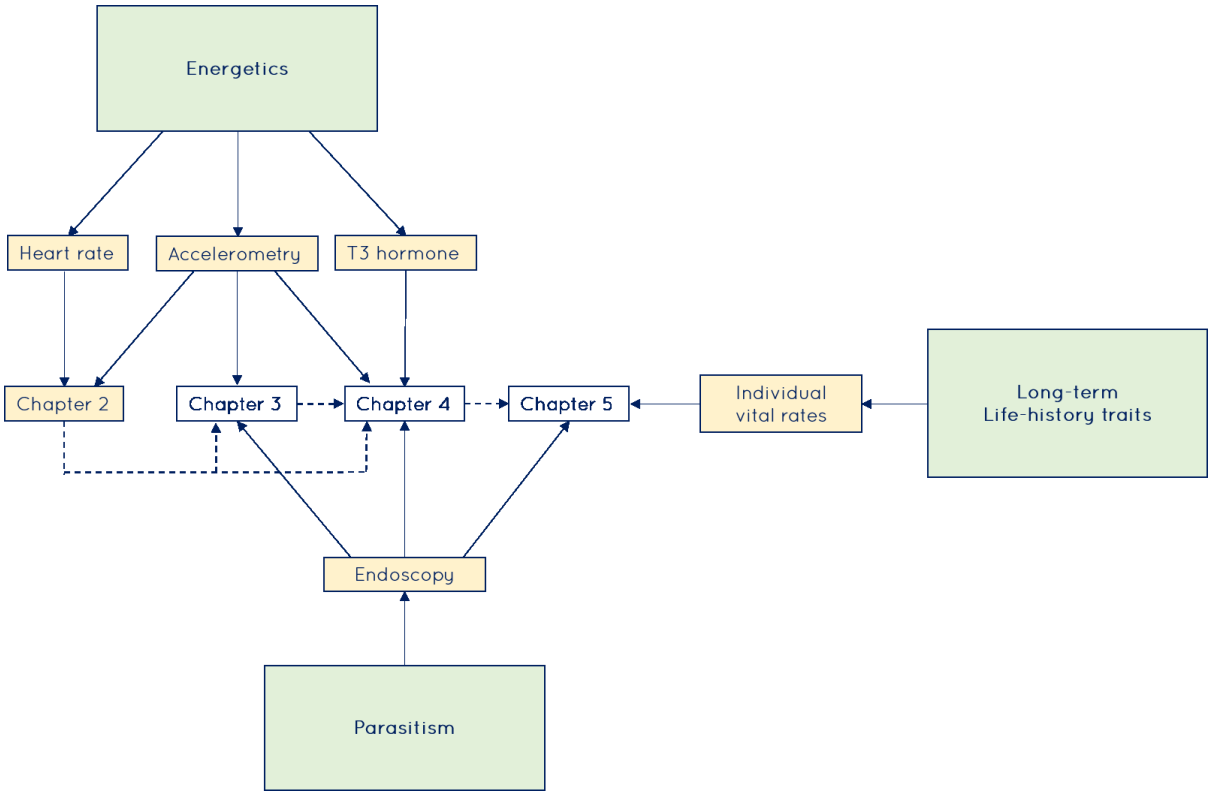

Figure 6. 1. Schematic of the different aspects of work contributing to this thesis

Novelty and impact

There is a large amount of literature and theory describing the importance of parasitism for evolutionary and ecological processes (Lochmiller and Deerenberg, 2000; Sheldon and Verhulst, 1996). However, the evidence for this in wild populations is equivocal. For instance, parasite removal increases fecundity for female reindeer, hares and house martins (Albon et al., 2002; Marzal et al., 2005; Newey and Thirgood, 2004) but parasites have no measureable detrimental effects to reproduction for white crowned sparrows and pied flycatchers (Siikamaki et al., 1997; Zylberberg et al., 2015). This means that parasite effects may be difficult to detect at certain temporal scales, or have varying importance across species. Importantly there is very little evidence of how or why these individual and population effects of parasites operate, despite their crucial importance to the mechanistic understanding of these processes.

What is novel about the work in this thesis is the use of individual natural parasite burdens and measurement of the impact of this on individual behaviour and energy use, as well as individual level breeding outcomes. These data are

very rare and collection has only been made possible due to the technological advances of data loggers (measuring energy expenditure), innovation of techniques (endoscopy to measure parasite load and hormonal proxy of RMR) and the tireless work of long term fieldworkers to collect detailed information of the breeding status of hundreds of individual adults and the continual ringing of chicks each year.

Immune response and physiological consequences

We found a positive relationship between a hormonal proxy for RMR (T3) and natural parasite load in female shags (Chapter 4). Immune response in birds has been estimated at 8–13% of BMR (Svensson et al., 1998) therefore higher RMR or maintenance costs are likely to reflect an elevated immune response. Experimentally elevated immune activity or stress responses have been shown to increase RMR in isopods, butterfly pupa, passerine birds and owls (Bize et al., 2010; Freitak et al., 2003; Lettini and Sukhdeo, 2010; Martin et al., 2003; Ots et al., 2001; Sheldon and Verhulst, 1996). However parasite removal or immunisation has also been shown to elevate RMR in ground squirrels (*Xerus inauris*) and chipmunks (*Tamias striatus*) (Careau et al., 2012; Scantlebury et al., 2007) and have no effect on BMR in blue tits (Parus caeruleus) (Svensson et al., 1998).

The high cost of immunity means that some infections may be allowed to persist if the costs of parasitism to the host do not outweigh the benefits of provoking an immune response to clear the infection (Peck et al., 2015; Sheldon and Verhulst, 1996). Immunity is therefore a subtle energy balance based on the severity of the infection. This means that a natural gradient of parasite load data is a powerful tool to understand the trade-offs of immunity and reproduction, as it provides higher resolution data on a continuous scale when compared to presence absence data collected from previous experimental studies.

Though this study provides clear evidence of higher maintenance costs in more highly parasitised individuals, it lacks the detail of the components that make up RMR. Different components of the immune system can vary in their costs to maintenance metabolism and may respond differently to energetic trade-offs (Lochmiller and Deerenberg, 2000; Peck et al., 2015; Raberg et al., 2002;

Svensson et al., 1998). Additionally, cell growth, tissue damage and repair costs which are not controlled for in this study may also contribute to variation in RMR, either through their association with parasite load or not. Therefore, to better understand the trade-off between activating an immune response versus the detrimental effects of parasitism it would be necessary to supress the immune response to parasitism. This could be done by experimentally suppressing immune pathways in wild animals in captive conditions.

An immune response not only entails short term costs associated with the activation of the response, but also involves long-term costs associated with oxidative stress and auto-immunity (Costantini and Møller, 2009; Hasselquist and Nilsson, 2012). These long-term costs might modulate energy investment in the immune response, or the trade-off between investment in immunity and reproduction. Though very few studies have quantified this cost (though see Hanssen (2006), Marzal et al. (2007)) it is an important consideration for understanding the impacts of parasitism on population dynamics and lifetime reproductive success. While the dataset of natural parasite loads in the Isle of May study is extensive, to test this would require long-term longitudinal data on immune activity, parasite load, survival and reproduction.

The role of parasitism in energy allocation

As introduced above, parasites have the ability to shift the balance of energy trade-offs through a complex series of costs. There are significant nutritional costs associated with the upregulation of an immune response as well as energetic costs associated with its maintenance (Lochmiller and Deerenberg, 2000). These increased costs can also draw resources away from other functions, and parasites themselves can take energy and resources from their host (Norris and Evans, 2000a). Thus, the consequences of the costs of parasitism described in this thesis depend heavily on the energy allocation of the host and how parasitism drive this.

Though we found a positive relationship between RMR and parasite load in females, there was no associated relationship between RMR and DEE. Additionally, shags with high parasite loads and high flight costs reduced the proportion of flight time per day. However, the reduction in flight time was more

than expected based solely on the increased cost of this behaviour. The extra reduction in flight is likely to compensate for the parasite associated increased RMR. Together these results suggest an energy ceiling and use of the 'allocation' energy management strategy in this species. So they cannot, or choose not to, increase their daily energy expenditure. This is in accordance with a number of seabird studies (Blackmer et al., 2005; Elliott et al., 2014b; Welcker et al., 2010) but contrary to other studies (including those of other seabird species) which provide evidence for both performance and independent strategies (Chastel et al., 2003b; Portugal et al., 2016).

More recent cross-species examination suggests that the energy management strategy observed is likely to be both species and context dependant, with an allocation strategy more likely to be observed under conditions of energetic stress (Halsey et al. In review). Shags and cormorants are interesting study species to examine immediate energetic trade-offs as they store very little fat, therefore have little access to an energy reserve to buffer any increase in energy use (Enstipp et al., 2005). Unlike most seabirds they seem to have a live-fast-die-young life-history strategy. They can have up to four young each year and their populations can be influenced dramatically by stochastic environmental events (Bogdanova et al., 2014b; Frederiksen et al., 2008; Wanless and Harris, 1997). These factors may be central in driving their energy use and forcing the use of the allocation energy management strategy. Therefore, an interesting consideration would be to understand how trade-offs vary in species such as seals and penguins which have fat storage but operate in similar environments and how their energy management varies temporally with varying energy requirements such as lactation or egg creation. For instance in species that store fat, trade-offs may operate at longer time scales, as increases in energy expenditure may be more feasible (Peck et al., 2015).

There is an increasing understanding that energy management strategy employed by a species or individual may vary in time depending on different constraints experienced or their ability to make use of resources available (Drent and Daan, 1980; Elliott et al., 2014a; Moe et al., 2009). However, increases or trade-offs in energy use are always going to be associated with fitness

consequences, whether in the short-term or life-time scale. Understanding energy strategies of different species allows us to understand the consequences of stressors to those individuals. However, it is what drives individual variation in energy use and allocation which ultimately leads to variation in population success. Energy is limited over a lifetime and increases in metabolic rate can cause oxidative stress. Therefore, though energy allocation trade-offs can have immediate or short-term consequences for individuals, the ability to increase expenditure in response to a stressor may incur long-term fitness effects (see Figure 6.2 for more detailed example). This can be difficult to measure over a life time due to the complications of other factors such as mass loss and gain that contribute to variation in energy use (Peck et al., 2016).

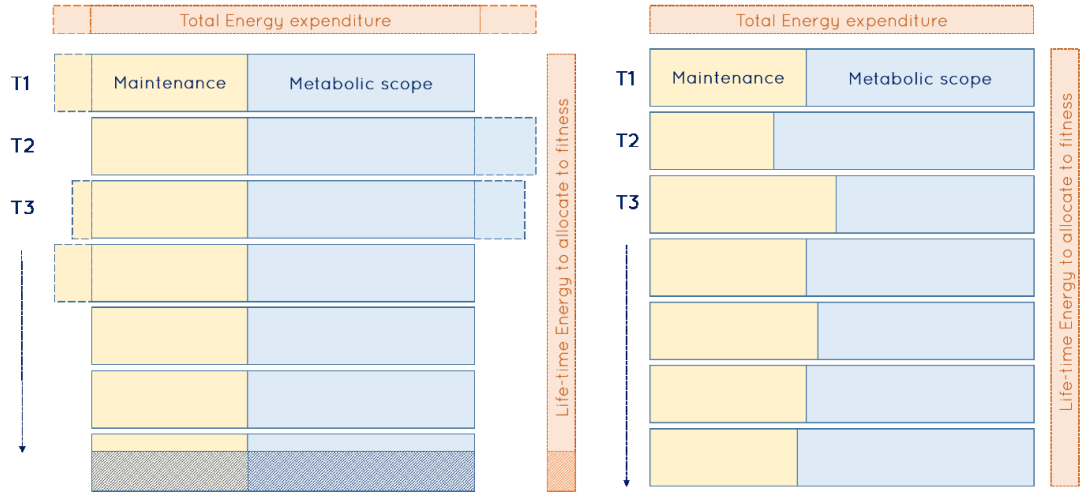

Figure 6.2. Energy management at different temporal scales. Energy allocated to either maintenance or metabolic scope or both may increase, which will cause an increase to total energy expenditure (A). Alternatively, energy allocated to maintenance and metabolic scope could be traded-off against one another (as in B) thereby maintaining total energy expenditure. Scenario A experiences no trade-offs between maintenance and metabolic scope in the present due to increased energy expenditure, however as increasing energy expenditure is known to cause oxidative stress which can increase physiological senescence and decrease survival, thus reducing the life-time energy allocated to fitness. In scenario B despite there being energy trade-offs in the present this works to keep total energy expenditure constant and as a result there is no additional reduction to life-time energy to allocate to fitness.

Measuring glucocorticoids (in particular Corticosterone (CORT)) could help understand the associated stress of energy balance between self and reproduction. CORT plays an important regulatory role in baseline levels of

stress (Sorenson et al., 2017) but is also involved in the modulation of parental investment meaning that it could mediate the energy allocation trade-off between a parasitism response and reproduction (Bonier et al., 2009; Wingfield and Kitaysky, 2002).

Behaviour specific responses to parasitism

We also found a behavioural and energetic cost of parasitism, where the cost of flight was higher in more highly parasitised females and these individuals reduced the duration they spent in flight to compensate for this increased cost. This gives us a novel mechanistic insight into the link between metabolism and behaviour and the ways in which parasites impact their host's fitness. Female shags forage further from their breeding colony and for longer than males and are more affected by wind (Bogdanova et al., 2014a; Lewis et al., 2015; Soanes et al., 2014). They also increase their foraging effort as brood age increases (Hicks et al., 2018; Reed et al., 2008b). With the additional constraint of parasitism on flight duration per day, highly parasitised individuals may have reduced foraging success. Thus, the ability to obtain enough food to provide their offspring appears to be compromised. Constraints on prey gain for central-place foragers such as seabirds are well known to have a direct impact on breeding success (Drent and Daan, 1980; Pichegru et al., 2007). A more detailed examination of the accelerometry data may be able to further determine if foraging success changes as a result of the reduction in flight time. Using changes in wing stroke frequency in this species can provide a method of obtaining information on foraging effort and success (Sato et al., 2008).

Though we do not know the definitive mechanism of the increase in flight costs with parasitism, one hypothesis is that feather quality could be reduced due to the costs of parasite related immune response as in house sparrows (Pap et al., 2013). An increase in one cost to maintenance is therefore being traded off with other maintenance traits causing knock on costs to flight behaviour. However investigating this hypothesis in shags is difficult due to their complex moult (Potts, 1971).

Contrary to expectation, at the scale of the behavioural bout the cost of diving behaviour in females was negatively related to parasite load. This could be due

to changes in behavioural complexity of dive bouts. Prey resources tend to be distributed patchily (Sparrow, 1999; Weimerskirch, 2007) and under these conditions high complexity foraging behaviour can enhance the probability of resource encounters, as opposed to more random movements (Macintosh et al., 2013). However, stress or immune response to parasitism have been found to compromise behavioural complexity in several species e.g. primates, sheep and ibex (Alados et al., 1996; Burgunder et al., 2017; Burgunder et al., 2018; MacIntosh et al., 2011). Complexity in diving is expected to be adaptable to maximise intake of prey, but is also energetically costly (Meyer et al., 2017). Therefore individuals with higher parasite loads may be foraging with less complexity but at lower energetic cost, but with the consequence of lower foraging success (MacIntosh et al., 2011). Diving bouts could be further analysed using fractal analysis to quantify complexity and associate this with parasite load (Macintosh et al., 2013) and the consequent energetic costs and gains.

The ability of an individual to extract resources from the environment determines the amount of energy available to be expended on fitness related activities (van Noordwijk and de Jong, 1986; Weimerskirch, 2018). Yet foraging itself is also costly and requires energy from the limited pool. Parasites draw energy away from activities due to the costs of the immune response, competing for resources with the host and infection (often associated with sepsis induced anorexia) (Lochmiller and Deerenberg, 2000). This creates an antagonistic feedback between foraging and parasitism. More energy intake is required to compensate for the increase in energy use, yet foraging costs are increased with parasite load. This work demonstrates that shags act under an allocation strategy during reproduction, a conservative strategy (Chapter 4). However, other species, or indeed shags under less constrained conditions, could work harder to increase intake.

Sex differences

Whilst both sexes increased energy expenditure as brood age increased, we consistently found sex based differences in the response of shags to their parasite load. This manifested as an energetic, behavioural and reproductive

impairment due to parasitism in females but not males. One explanation for sex-specific differences in relation to parasitism is that males possess smaller immune defence organs (Møller et al . 1998) and mount weaker humoral and cellular immune responses in comparison with females (e.g. Grossman 1985; Moreno et al . 2001; Fargallo et al . 2002). However, this theory would be likely to predict a larger cost of parasitism to males, and our results are contrary to this. However our work is consistent with previous experimental evidence in this species showing the effect of parasitism on reproductive output in females but not males (Reed et al., 2008b).

Male shags and cormorants are known to dive deeper and for longer than females, when foraging, as their larger size relates to a lower mass-specific metabolic rates (Cook et al., 2013). This makes males more efficient foragers in a given environment meaning they may have more flexibility in terms of energy use and gain. Males out compete females due to interference or indirect competition and drive them offshore. Hence, female shags at the Isle of May forage further from shore and thus have a higher commuting costs than males (Bogdanova et al., 2014b) which adds to their higher daily energy expenditure. This is potentially why they experience a larger effect of wind on their foraging behaviour than males (Lewis et al., 2015). In general females consistently make greater investment than males in reproduction (Clutton-Brock, 1991; Cole, 1954; Trivers, 1972) and may consequently experience stronger energetic trade-offs than males (Hicks et al., 2018; Korpimaki et al., 1993; Reed et al., 2008b). These sex differences in investment, exacerbated or driven by the impact of parasites could have consequences for survival; indeed, previous work has shown that during winter female shags have lower survival than males (Brooks et al., 2002). This has been demonstrated in other studies which show that increased work during reproduction increases mortality (Boonekamp et al., 2014; Daan et al., 1996)

We also found sex-specific age effects where males showed a quadratic relationship between age and breeding success. This occurs in a number of wild vertebrate populations and is well documented in seabirds (Clay et al., 2018; Froy et al., 2013; Froy et al., 2017; Murgatroyd et al., 2018b). These age effects

found in males could be in part linked to their response to parasitism. We found no evidence for a differential cost of parasitism to maintenance or breeding success in males. However, by definition parasites are costly to the host. Males may bear the cost of parasitism rather than invest in an immune response but this may cause long-term somatic damage (Hasselquist and Nilsson, 2012), contributing to their decline in breeding success in later life. We also found a decline in RMR with age in males but not females. This has been reported in a number of other seabird species (Blackmer et al., 2005; Moe et al., 2007) as well as other species (for review see Elliott et al. (2014b)) and is likely due to strategic restraint. However, this is a cross sectional study, therefore this may be explained by the higher survival of those individuals with lower maintenance costs rather than a decline in maintenance costs with age. Either way, these sex differences demonstrate that different trade-offs exist in males and females that can vary temporally. Where this work is limited is understanding the lifetime consequences of parasitism. Though they were not determined in this study, crucially the sex differences we found in the effects of parasitism in this thesis may have significant implications for selection and population processes. For example, given males tend to have stronger senescence effects but often lack immediate effects of parasites (including no effect of parasitism on breeding success in this study) it would be interesting to examine what role parasitism has in the aging process for males. Again, this work would involve long-term data on parasitism and survival and a physiological mechanisms such as reduction in telomere length.

Lifetime consequences and trade-offs

When energy is limited, and self-maintenance costs increase with parasite load, the work in this thesis suggests that there is likely to be a trade-off in terms of the amount of energy allocated to reproduction. Thus, when foraging costs increase with parasite loads, individuals may be constrained in their reproductive investment. As such this may constrain the ability of mothers to respond to the additional strain of feeding a growing brood. We found a 30% reduction in fledgling success in females across the range of natural parasite burden, a large reduction in reproductive output for the host. What is significant

about this finding is that the parasite effect on breeding success is not dependent on the environmental conditions. Previous studies have suggested that the impacts of parasites are condition dependant and only experienced in poor conditions. (Bize et al., 2010; Laaksonen et al., 2002; Reed et al., 2008b; Tompkins et al., 2011). However, the data presented here encompass seven years of data collection with varying conditions, in which we found no interaction between parasitism and prevailing environmental conditions. This suggests a crucial role for parasites in population dynamics. As long-term parasite studies are rare in wild populations this finding may be important in raising the profile of including the effects of parasitism in ecological studies.

In addition to the negative relationship found between parasitism and fledgling success, there may also be impacts of adult parasitism on other fledgling traits such as size. Reed et al (2008) showed that females relieved of their parasite load were able to raise more sons, as males are 20% larger than females and require more food as chicks. Smaller seabird fledglings typically have less probability of surviving to reproduction (Arnold et al., 2006), meaning that there may be an additional hidden cost to the population. Shag chicks themselves also have a high prevalence of parasite infection (Granroth-Wilding et al., 2016) and this could interact with adult infection levels. Chick growth success can be mediated by their own parasite load and the environment (Granroth-Wilding et al., 2014). Thus, both reduced provisioning due to adult parasite load and limited growth via chick parasite mediated effects could decrease offspring survival or quality, all of which contribute to the fitness of the parent.

Understanding both the effects of parasites on breeding success and the mechanism by which it operates provides us with a powerful ability to model the impact of parasitism on the population. This is a crucial next step to this work, as it will help incorporate what we know about individual effects, and be able to quantify these impacts to the population. As parasitism effects were found to be additive to other drivers of reproductive success, a key vital rate, it is important to include this cost and driver of individual success. Mechanistic models have a greater capacity to predict beyond current ecological conditions

than correlative models, which is a key advantage when predicting the outcomes of rapid environmental change (Tomlinson et al., 2014).

Using energy as a currency also enables comparisons to be made across species on the same scale, to answer questions about species that interact in the same energetic landscape. For instance, energetic fitness, based on the reproductive power of an individual was first proposed by Brown et al. (1993) to describe the rate of energy acquisition of an individual and rate at which these resources are transformed into offspring (Brown et al., 1993). Though this concept has existed for years it is not been put into practice in many wild studies (though see (Grémillet et al., 2018)). Yet this framework could help take a broader ecosystem approach to questions.

Parasite acquisition and diet

We have found and quantified important costs and consequences of parasitism to individuals in this population, yet remarkably we do not know the factors that determine variation in parasite load. One possibility is diet, which we know has changed and diversified over the last 30 years in this population (Howells et al., 2017) and different diet items could vary in their parasite loads. Therefore, it is likely that heterogeneity in individual diet may have an effect on parasite load. Alternatively intrinsic differences in immunity may play a role in determining individual variation in parasite load (Lochmiller and Deerenberg, 2000). However, diet could also interact with the intrinsic immune function of individuals, for instance poor quality individuals may forage in low quality patches on highly parasitised fish and have low immune ability to fight the costs of parasitism.

Communities of parasites can interact with one another (Telfer et al., 2008). Yet in this thesis we only measure one macro-endo-parasite rather than the full diversity of parasites. This is mostly due to the methodological constraints of directly measuring parasite load in wild populations, and in this particular instance we are able to accurately quantify the nematode worm *Contracaecum rudolphii*. However, it is important to consider that parasites may interact with one another to affect host success in complex ways (Pedersen and Fenton, 2007).

Conclusion

Energy is a crucial currency in life but little is known about how animals make energetic decisions. This thesis presents novel work on the way in which parasitism drives variation in energy allocation and use at an individual level. Parasites infect nearly every known animal taxon (Dobson et al., 2008), thus it is important to quantify their impacts on host fitness and population success. We show that the energetic constraint of parasitism has fitness consequences to their hosts through a reduction in female breeding success, in a wild population of seabirds. The findings emphasise the importance of natural parasite loads in shaping the ecology and life-history of their hosts, which can have significant population level consequences.

Using an energetics approach, we further investigated the mechanism behind these fitness effects. To do so, we quantified the role of parasitism in mediating energy allocation between reproduction and self-maintenance and found a positive relationship between maintenance costs and parasite load in females. Consequently, we also demonstrate that parasite load is related to increased energetic costs of flight in females and a reduction in the time spent in this behaviour (Hicks et al., 2018).

This work relied heavily on new techniques and technology as well as creating new techniques, which are enabling this area of ecophysiology to grow (Elliott, 2016). In order to carry out this work we outlined an approach to generate behaviour-specific estimates of energy expenditure from ODBA, which can be used to more accurately estimate total energy expenditure in the complex behaviour of free-living cormorant species. This thesis is part of a movement to use a more mechanistic and physiological approach to answer ecologically relevant and important questions (Tomlinson et al., 2014). This is particularly important in realising and quantifying the multifaceted effects of parasitism on individuals in wild populations. The work of this PhD demonstrates that energetics is a powerful framework to aid the understanding of individual-level mechanisms driving life-history.

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